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Editorial

Dear Researchers and Readers

Amidst the COVID-19 pandemic and the unprecedented lockdown so as to contain the spread of the novel coronavirus, academic activities have been confined to homes with e-learning initiatives and technology driven education being adopted. It is understandable that initial confinement led to worries and anxieties but gradually we adapted to the ‘New Normal’, as it is called now, and would be for a pretty long time to come. Despite, the prevailing situation, the number of manuscripts received during this period for consideration of publication in The NEHU Journal was very encouraging. I must place on record the time and effort given by the reviewers and prompt action of the authors in revising the manuscripts. The editorial team was always there to assist whenever I needed. As the Journal publishes articles of multidisciplinary nature, the present volume too has a mix of articles drawn from Life Sciences, Physical Sciences, Human & Environmental Sciences and Social Sciences. The present volume is presented as a combination of two issues of the journal i.e. the January-June issue (No.1) and the July-December issue (No.2) usually published as separate issues.

The contents of this volume begins with the article on **“Index of opportunity for natural selection among the Idu Mishmi of Dibang Valley district, Arunachal Pradesh”** by D. K. Limbu and Dolly Gogoi who highlight the index of selection intensity in the Idu Mishmi, a homogenous tribal group inhabiting the Dibang valley district of Arunachal Pradesh and compare the findings with the related information from the published data on other mongoloid populations of Northeast India.

Anamika Upadhaya and co-authors present the **“Effect of temperature on germination of Citrus macroptera, Citrus latipes and Citrus indica seeds”** which is a crop of high economic importance cultivated extensively in Northeast India.

Taranga Jyoti Baruah and Lakhon Kma in their article **“Flavonoids and radiation response of cancer cells: A therapeutic prospective”** present how radioprotectors and radiosensitizers can increase the effectiveness of radiation therapy and Flavonoids can act both as radioprotectors and radiosensitizers. As radioprotectors, flavonoids could up-regulate the anti-oxidant enzymes and DNA-repair processes in the cells and as

radiosensitizers, flavonoids inhibited the pro-survival pathways while up-regulating the apoptotic pathways.

In their research findings on **“Nutritional status in relation with age and place of residence among the adult Rongmei Naga of Manipur”**, Guangdikhuanliu Riamei and Sudipta Ghosh highlight the association between nutritional status, through Body Mass Index (BMI), and age and place of residence (rural vs. urban) among the adult Rongmei Naga of Manipur.

The article **“A proteomic approach to pesticide stress management in microalgae”** by Ng. Kunjarani Chanu and Neha Chaurasia highlights the proteomics approach using Two-dimensional gel electrophoresis and MALDI-TOF for analysing the protein profile of microalgae and the importance such molecules in combating pesticides stress.

Sumita Biswas and her group have presented their findings on **“Isolation and identification of cellulose degrading bacteria from gut of two herbivorous pest larvae”** along with the importance of insect gut microflora who contribute towards the utilization of complex food resources in the gut of the host.

In the article **“Agro-forestry: An alternative for Jhum Cultivation in Meghalaya”**, Lolita M. Shangpliang presents a lucid information on Agro-forestry as an alternative to jhum cultivation highlighting the details about the proposed methods of Agro-forestry.

Jayanta Kumar Behera in his paper **“Migration of tribals and their settlement: A study in Dindori district of Madhya Pradesh”** has presented how pulling as well as pushing factors induce tribal migration in different parts of central India with a focus on the migration pattern of tribal population in Dindori district of Madhya Pradesh.

The article **“Evaluation of Antioxidant properties of Quercetin in DEN-induced Hepatocellular Carcinoma in BALB/c mice”** by Casterland Marbaniang and co-authors present the research findings on the antioxidant properties of Quercetin in diethylnitrosamine (DEN)-induced Hepatocellular Carcinoma (HCC) where HCC is one of the most common malignant tumors in the world.

Rik Ganguly and Atanu Bhattacharjee in their article **“Atherosclerotic plaque development: Disease Pathogenesis and emerging treatment options”** present a picture on basic mechanism that helps in Artherogenesis which are trans-migration of different granulocytes in the intimal layer of the artery with the help of adhesion molecules expressed in the outer layer of the activated endothelium.

Satyawada Rama Rao and co-authors present the scientific relevance and significance of **“Okra- an important vegetable crop of India”** as a major vegetable crop of India having popularity among farmers and consumers because of its diverse usage as fiber yielding and ornamentals for their beautiful flowers.

“A preliminary note on big data and machine learning technologies” by Sandeep Sharma and N.C. Barwar narrate on Big data as high-volume, high-velocity and variable information assets which demand cost-effective, innovative forms of processing for enhanced insight and decision making, and many machine learning strategies are coordinated with big data analytics tools.

Before I close, I once again thank the reviewers for the precious time spared and their promptness in reviewing the articles. It is my earnest request to the scholars to submit their articles for publication in the future issues of The NEHU Journal.

Stay safe

Prof. S.R. Joshi

Editor

Index of opportunity for natural selection among the Idu Mishmi of Dibang Valley district, Arunachal Pradesh

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Abstract

The index of opportunity for selection was calculated to understand the nature and extent of natural selection operating in the Idu Mishmi population. The main objective of this study is to estimate the index of selection intensity in the Idu Mishmi, a homogenous tribal group inhabiting the Dibang valley district of Arunachal Pradesh and further the findings of the present study is to compare with the related findings obtained from the published data on other mongoloid populations of Northeast India. Considering the range of Indian population, the index of total selection intensity (I) calculated in the present population was found to be very low. It seems that in shaping the genetic make-up of the Idu Mishmi population, natural selection is operating with very low intensity and it contributes more through differential mortality than differential fertility. Low socio-economic status of the people and difficulties in percolating the medical facilities up to the grass root level could be the probable reason for higher contribution of mortality for the evolution mechanism of the present population through natural selection.

Keywords: Fertility, mortality, index of total selection intensity, Darwinian fitness, Idu Mishmi

Introduction

Natural Selection is a major evolutionary force, which can bring about changes in the genetic make-up of a population over generations. In order to understand how selection is operating in this population, the index of opportunity for selection has been calculated according to the formula given by Crow (1958) as well as by the modified formula of Johnston and Kensinger (1971). It is well-understood that fertility and mortality are the two vital events through which natural selection operates. In the given environment, the function of these two demographic variables operates singly or jointly to determine fitness (Darwinian fitness) of a particular population. The fitness of a population is measured through selection intensity which is expressed in terms of differential fertility and differential mortality that the birth and death rates are all selective. Variables such as age-specific fertility and mortality bring changes in the intensity of natural selection. Thus, several studies have suggested that the above demographic components are directly responsible for the rate and direction of human evolution (Wright, 1943; Cavalli-Sforza

et al., 1966).

It is well-known that selection is a major evolutionary force that can bring about changes in the genetic make-up of a population. Crow (1958) was first to interpret the Fisher's fundamental theory of Natural selection in human population by using demographic data. Taking into account the differential fertility and differential mortality, Crow proposed the index of total selection intensity (now the index of opportunity for selection). In order to understand how selection is operating in human population, the index of opportunity for selection has been calculated according to the formula given by Crow (1958) as well as by the modified formula of Johnston and Kensinger (1971). Since then, a large number of studies have been carried out on various populations of the world to demonstrate the role of natural selection in imposing characteristic fertility and mortality in them (Murty and Ramesh, 1978; Tripp-Reimer, 1980; Rao and Murty, 1984; Jorde and Durbize, 1986; Basu *et al.*, 1988; Sengupta, 2004; Kapoor and Kaur, 2012; Sarma, 2013).

Objective

The objective of the present study was to estimate the index of selection intensity (opportunity for selection) in the Idu Mishmi population and also to compare the present findings with those existing on other populations, particularly with those reported for the mongoloid populations of Northeast India. Further, Dibang valley district, the home for the Idu Mishmi has the lowest population density in the entire country (1person / km²) (Census, 2011). Information on some related demographic parameters were also collected with the idea that these data might throw some light in understanding both genetic composition and extra-somatic factors influencing the study population.

Materials and Methods

The present study is based on a fieldwork conducted among the Idu Mishmi, one of the major tribal populations of Dibang Valley district of Arunachal Pradesh. A total number of 103 married women were randomly interviewed under the present study. Demographic data were collected through in-depth interview with each of the married women using structured fertility schedule following recommendations of the World Health Organization (1964 and 1968) which includes data on individual records, fertility, mortality and marriage pattern. Data collected on fertility and mortality was as follows: number of conceptions, number of live births, birth order, age, sex and marital status of each offspring, number of dead children, age at death, causes of death and reproductive wastage (abortions and still births).

Data analysis

For analyzing fertility, data on the four important measures such as mean number of live-births and surviving children to all married women of all ages, child-woman ratio, completed family size, and total fertility rate were collected which are the important measures on fertility. Similarly for mortality, the parameters included were infant mortality

**Index of opportunity for natural selection among the Idu Mishmi of
Dibang Valley district, Arunachal Pradesh**

(death before 1 year of life); juvenile or premature mortality (death before 15 year of age) and reproductive wastage. Crow's formula (1958) and the modified formula suggested by Johnston and Kensinger (1971) were applied to calculate the index of total selection intensity. Since fertility declines drastically after 40 years of age, only those mothers who had reached 40 years and above were included in the present study. The parameters used in calculating selection intensity of the study population are presented in Table 1.

Table1. Parameters used in calculating total selection intensity

Parameter	Frequency
Number of mothers age 40 years and above	46
Number of reported pregnancies	248
Number of live-births	235
Number of surviving children	355
Number of deaths before 15 years	25
Number of embryonic deaths	13
Proportion of survivors to birth(P_b)	0.9476
Proportion of child death (i.e., deaths before 15 years)(P_d)	0.1064
Proportion of embryonic deaths(P_{ed})	0.0524
Mean number of live-births per mother of completed fertility (i.e., ≥ 40 years)($\pm \bar{x}$)	5.1087
Variance in the number of live-births due to fertility, (V_f)	2.6186

In order to estimate the total selection intensity, two statistical formulae viz., formula proposed by Crow (1958) and subsequently modified by Johnston and Kensinger (1971) have been applied by taking into consideration the prenatal mortality.

Crow's index is separated into two components, known as the index due to fertility and the index due to mortality. Then, Crow has combined the two indices to calculate the index of opportunity for selection, which is as follows:

Crow's index:

$$I = I_m + (I_f / P_s)$$

$$I_m = P_d / P_s$$

$$I_f = V_f / (\bar{x})^2$$

$$P_s = 1 - P_d$$

where, I is the index of total selection intensity; I_m is the index of selection due to infant mortality; I_f is the Index of selection due to fertility; \bar{x} is the mean number of live births per women of completed fertility (i.e., ≥ 40 years); V_f is the Variance in

the number of live-births due to fertility; P_d is the Proportion of premature deaths (deaths before 15years of age); and P_s is the Proportion of survivors ($P_s = 1 - P_d$).

Johnston and Kensinger's index:

The above formula, proposed by Crow (1958), has been modified by Johnston and Kensinger (1971). They have taken into consideration prenatal mortality and suggested the following formula:

$$I = I_{me} + I_{mc} / P_b + I_f / P_b P_s$$

$$I_{me} = P_{ed} / P_b$$

$$I_{mc} = P_d / P_s$$

$$I_f = V_f / (\bar{x})^2$$

where, I is the index of total selection intensity; I_{me} is the index of selection due to embryonic mortality (i.e., prenatal mortality); I_{mc} is the index of selection due to child mortality (i.e., mortality before 15years of age); I_f is the index of selection due to fertility; \bar{x} is the mean number of live births per women of completed fertility (i.e., ≥ 40 years); P_{ed} is the proportion of embryonic deaths (i.e., prenatal deaths); $P_b = 1 - P_{ed}$ is the proportion of survivors to birth; P_d = proportion of child deaths (i.e., death before 15 years of age); V_f is the variance in number of live births due to fertility; and $P_s = 1 - P_d$ is the proportion of survivors i.e., birth to reproductive age.

Results and Discussion

The results of the analysis of fertility and mortality data of 46 women who have completed their reproductive lifespan are presented in Table1. This table depicts the parameters used in calculating the indices of total selection intensity according to the Crow's and modified formula of Johnston and Kensinger. Table1 shows that there were 46 mothers aged ≥ 40 years. The mean number of live births to such mothers was recorded to be 5.1087 with the corresponding variance of 2.6186. The proportion of child mortality before reproductive age and the proportions of embryonic deaths were 0.1064 and 0.0524 respectively.

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Dibang Valley district, Arunachal Pradesh**

Table 2. Parameters used in calculating total selection intensity among four mongoloid populations of Northeast India

Parameter	Populations			
Frequency	Idu Mishmi (P r e s e n t study)	Mishing (S a r m a 2013)	Minyong (S a r m a 2013)	Semsa (G h o s h and Limbu 2000)
Number of mothers age above 40 years and above	46	77	74	80
Number of reported pregnancies	248	554	561	537
Number of live-births	235	462	487	521
Number of surviving children	355	387	378	138
Number of deaths before 15 years	25	75	109	167
Number of embryonic deaths	13	92	74	17
Proportion of survivors to birth(Pb)	0.9476	0.8377	0.7762	0.9634
Proportion of child death (i.e., deaths before 15 years of age)(Pd)	0.1064	0.1623	0.2238	0.3205
Proportion of embryonic deaths(Ped)	0.0524	0.1661	0.1319	0.0366
Mean number of live-births per mother of				
Completed fertility(i.e., ≥ 40 years) $\pm \bar{x}$	5.1087	6.00 \pm 0.31	6.58 \pm 0.33	6.5125
Variance in the number of live-births due to fertility,(Vf)	2.6186	3.03	4.67	4.1748

Table 2 shows the parameters used in calculating the index of total selection intensity among the four mongoloid populations of Northeast India. It may be noted that the average number of live births per mother of the Idu Mishmi is found to be 5.1087, which is much lower than rest of the tribes compared viz., the Mishing (6.00 \pm 0.31), Semsa (6.5125) and the Minyong (6.58 \pm 0.33). Similarly the proportion of embryonic deaths (abortion and stillbirth) is also very low in Semsa (0.0366) and the Idu Mishmi (0.0524) compared to the Mishing (0.1661) and the Minyong (0.1319). Moreover, in respect of the proportion of premature deaths up to pre-reproductive age, (i.e., deaths before 15 years of life) the present population shows lowest value (0.1064) when compared with the Mishing (0.1661), Minyong (0.2238) and the Semsa (0.3205). However, it is interesting to note that in respect of the proportion of survivors up to 15 years of age and above, the Semsa (0.9634) and Idu Mishmi (0.9476) show higher values than the Mishing (0.8377) and the Minyong (0.7762). Table 3 presents data on the demographic variables i.e., fertility and mortality of the Idu Mishmi population which are applied for determining their selection intensity.

Table 3. Fertility and mortality data following parameters recommended by WHO (1964 and 1968)

			Fertility				
Marriage age at Marriage ±SE(Years)	Marriage age at firstchild birth ±SE(Years)	Fertility ratio/100 women	Completed family size				TFR
			Average no. of live births/ mother	Average no. of surviving children/ mother	Average no. of live births/ mother of present age	Average no. of surviving children of present age group	
Male: 25.56 ±0.23	27.88 ±0.37						
Female:20.07 ±0.23	22.17 ±0.15	37.43	5.11	4.57	3.86	3.45	3.9423
Mortality(infant, juvenile and reproductive wastages)							
Parameters							
Total number of mothers							103
Total number of pregnancies							416
Total number of live births							398
Total number of deaths before 1year of age							25
Total number of abortions							15
Total number of still births							6
Total number of reproductive wastage							21
Death before 1year of age (%)							6.28
Death between 1and 4 years of age (%)							0.50
Abortion (%)							3.60
Still birth (%)							1.44
Reproductive wastage (%)							3.04
TFR: Total Fertility Rate; SE: Standard Error; WHO: World Health Organization							

To find out the nature and extent of natural selection operating in the Idu Mishmi population, the index of total selection intensity was calculated by considering that certain differences in fertility and mortality are heritable. For this purpose, methods given by both Crow (1958) and Johnston and Kensinger(1971) were applied as given in Table 4.

According to Crow's formula, the values of I , I_f and I_m were 0.2067, 0.1003 and 0.1191 respectively. When the selection intensity was calculated using the modified method of Johnston and Kensinger; the I_{me} , I_{mc} , I_f and I , the respective values were found to be 0.553, 0.1191, 0.1003 and 0.2535.

Based on all these indices, the total selection intensity I calculated according Johnston and Kensinger's formula was found slightly higher than that found according to Crow's formula as the embryonic mortality was included in the modified formula of the former.

However, by using both the methods, it still showed that natural selection was acting with very low intensity on this population and was operating more due to differential mortality than due to differential fertility.

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Table 4. Indices of Selection intensity

According to Crow (1958)			According to Johnston and Kensinger (1971)			
I_m	I_f	I	I_{me}	I_{mc}	I_f	I
0.1191	0.1003	0.2067	0.0553	0.1191	0.1003	0.2535

Cavalli-Sforza and Bodmer (1971) reported that among most agrarian and tribal societies, mortality contributes more towards selection rather than fertility which supports the findings of the present study. Similarly, studies on many Indian populations indicate that deaths prior to pre-reproductive age contribute more to the process of natural selection. The successful adaptation of a group inhabiting to its environment is reflected by its fertility and mortality rates. For example, a higher mortality rate may be indicative of poor environmental conditions besides influence of extra-somatic factors such as poor nutrition, large family size, unhygienic living conditions, higher incidence of diseases and lack of proper medical facility. Thus, it is not only the genetic composition of a population group, but extra-somatic factors that can also contribute to the fluctuating values of the selection intensity.

Table 5. Indices of Selection Intensity among some populations of Northeast India

Population (N)	Crow's index (1958)			Source
	I_m	I_f	I	
Idu Mishmi (46)	0.1192	0.1003	0.2067	Present study
Bod-Kachari (NA)	0.110	0.130	0.250	Guha and Mukherjee (1990)
Sonowal- Kachari(72)	0.1676	0.1638	0.3589	Sengupta and Kalita (1996)
Khampti (29)	0.179	0.113	0.312	Sarkar <i>et al.</i> (1994)
Mishing (77)	0.1937	0.0842	0.2942	Sarma (2013)
Minyong(74)	0.2883	0.1078	0.4272	Sarma (2013)
Gallong (36)	0.750	0.180	0.170	Chakravarty and Ahmed (1989)
Singpho(143)	0.437	0.406	1.020	Padmanabham and Jaswal (1982)
Muklom(40)	0.107	0.188	0.315	Sarkar (1997)
Apatani(Guth)(120)	0.440	0.312	0.889	Padmanabham and Jaswal (1982)
Apatani (Guchi)(64)	0.435	0.195	0.710	Padmanabham and Jaswal (1982)
Semsa (80)	0.471	0.0984	0.6165	Ghosh and Limbu (2000)

Figures in parentheses indicates number of women (≥ 40 years) : NA: Not available

Table 5 depicts the indices of selection intensity among some mongoloid populations of Northeast India. The values of I in the Idu Mishmi tend more toward the lower limit of the range. Except Gallong (0.170), the value of I found much lower in the present population (0.2067). A similar trend is observed with I_m and I_f which are at the lower limit of the range of Indian populations as reported by Reddy and Chopra (1990). In respect of I_m , the Idu Mishmi (0.1192) shows lower value than all the populations considered for comparison except the Boro-Kachari (0.110) of Assam and the Muklom (0.107) of Arunachal Pradesh. Other than the Mishing (0.0842) and the Semsu (0.0984), the value of I_f in the study population (0.1003) observed to be much lower than all the populations compared. Except the Bodo-Kacharis, in all the populations compared, the index of selection due to mortality component is contributing more to the total index of selection intensity. Further, it may be noted that selection is operating with very low intensity in the present population.

Reddy and Chopra (1990) have compiled all the results, reported on 96 Indian populations on the indices of selection intensity, calculated according to the formula of Crow. It is found that the index of total selection intensity varies from 0.258 in a subgroup of Yanadi tribe (Vasulu, 1987) to 2.250 in Kota (Basu, 1972). Comprising with the Indian range, it seems that the total selection intensity in the Idu Mishmi falls towards the lower limit of the range.

Conclusion

From the findings of the present study, it appears that selection is operating with very low intensity and contributes more through differential mortality than differential fertility. This trend might be due to poor socio-economic status and lack of proper health-care facility available to the study population. These factors have negative impact on the higher contribution of mortality for the evolution mechanism through natural selection. Livingstone and Spuhler (1965) have suggested that if the index of total selection intensity comes to zero, it means that there will be no change in the genetic make-up of a population through selection. Therefore, in the light of the present findings it can be concluded that the role played by natural selection in influencing the genetic variation of the study population cannot be ignored.

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Effect of temperature on germination of *Citrus macroptera*, *Citrus latipes* and *Citrus indica* seeds

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Abstract

Seeds are an important means of propagation of Citrus species. Seeds of three wild Citrus namely; Citrus macroptera Montrouz., Citrus latipes (Swingle) Tanaka and Citrus indica Tanaka were germinated at 20°C, 25°C, 30°C and 35°C temperature to observe the effect of temperature on germination. Mean germination time and percentage seed germinated were recorded and used to determine optimum temperature for germination. Viability of seeds determined using chemical and germination tests yielded similar results. Optimum temperature for germination was found to be 28°C for C. macroptera and C. latipes and 26°C for C. indica.

Keywords: Germination, wild, *C. macroptera*, *C. latipes*, *C. indica*, Meghalaya

Introduction

Citrus has been domesticated since ancient times, and where ‘natural’ populations are located, it is often difficult to determine whether they represent wild ancestors or are derived from naturalized forms of introduced varieties. Though relatively rare in wild, *Citrus* are mostly found as scattered trees in primary forests in remote areas rather than as pure stands. In India, a vast reservoir of *Citrus* diversity exists both in wild and in cultivated forms. North-eastern India is considered as natural home of many *Citrus* species with wide occurrence of indigenous species like *C. macroptera*, *C. latipes* and *C. indica* (Malik *et al.*, 2006). Though cultivated worldwide, some species of *Citrus* like *C. macroptera*, *C. latipes*, *C. indica*, *C. ichangensis* and *C. assamensis*, are still found in wild (Singh, 1981). Researchers have described this region as hot spot for *Citrus* biodiversity and have highlighted that erosion of these genetic resources is a cause of concern (Chadha, 1995; Singh *et al.*, 2001). *C. macroptera* and *C. latipes* are used by many traditional societies for their medicinal and culinary properties (Upadhaya, 2013; Upadhaya *et al.*, 2016). However, lack of cultivation of these species and loss of natural forest cover has underlined need to adopt complementary conservation strategies to ensure continued existence of these species in future (Malik *et al.*, 2006).

Seeds offer a convenient way to store germplasm over time and to transport it over long distances even across international boundaries (Boswell, 1961; Heydecker, 1972) and adequate seed germination is the key to successful tree establishment (Radosevich *et*

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al., 1997). Seeds are particularly valuable in *Citrus* culture because of their importance in the establishment of nursery stocks and because seeds are known to be relatively free from the common *Citrus* virus and fungal diseases (Kernick, 1961; Onwueme, 1978; Fawusi, 1989). Moreover, *Citrus* seeds are easily obtainable, relatively inexpensive, plentiful, and grow true-to-type (Castle, 1981; Rouse and Sherrod, 1996).

The effect of temperature on the germination of various commercially important species of *Citrus* has been studied by a number of researchers (Wiltbank *et al.*, 1995; Saipari *et al.*, 1998). However, such information is lacking on the fast diminishing wild *Citrus* species that have been used by tribal communities since ages. This study was conducted to determine the viability of the seed and its optimum temperature for germination.

Materials and Methods

Khasi Papeda (*Citrus latipes* (Swingle) Tanaka), Melanesian Papeda (*Citrus macroptera* Montr.) and Indian wild orange (*Citrus indica* Tanaka) are the three wild *Citrus* found in Meghalaya. They are culturally important species and are used in traditional healing system. The three species belongs to two subgenera, *Citrus* and *Papeda* (Spiegel-Roy and Goldschmidt, 1996), distinguished by leaf, flower and fruit characteristics. Seeds of these fruits were extracted from ripe fruits, rinsed thoroughly and soaked for 24 hrs in distilled water. These were then treated with sodium hypochlorite solution (0.1 %) and rinsed. As a general rule the fresh seeds as soon as they were removed from the fruit were used for the tests.

Seed viability test for these species was done using TTZ test given by Patil and Dadlani (2009) and compared to result of germination. Germination under different temperature was tested in the controlled condition in a Seed Germinator. Ten replicates of 10 seeds each in the wet filter paper was placed in Petri-dish and kept at 20°C, 25°C, 30°C and 35°C under light conditions of 16 hr light and 8 hr dark following Barton (1943). Daily records were made to observe the germination and the percentage of germination ($100 \times \text{number of seedlings emerged} \div \text{number of seeds planted}$) was calculated for each temperature.

Results

Seed viability test of the three species of *Citrus* using TTZ test showed an average of 90 % viability in *C. macroptera*, 91 % in *C. latipes* and 94 % in *C. indica*. The percentage seed germination of the three species of *Citrus* under different temperature is given in the Table 1. Germination in all three species increased with increase in temperature up to 30°C and decreased at 35°C. For *C. macroptera* and *C. latipes*, gradual increase in temperature enhances seed germination rate and decreases the number of days required for germination, whereas at 35°C temperature, the number of days required for germination also increased. While for *Citrus indica* unlike other two species, the number of days required for germination decreased at even 35°C.

ANOVA was performed to determine the effect of temperature on the percentage of germination and mean number of days required for the germination for all the three species. Variation was found to be statistically significant (Table 1).

Table 1: Germination percentage of three <i>Citrus</i> species at different temperature				
Species	Temperature (°C)	No of days		Percentage germination
		Range	mean number of days	
<i>Citrus latipes</i>	20	10-20	15	12***
	25	9-20	14	81***
	30	7-20	13	92***
	35	15-25	20	38***
<i>Citrus macroptera</i>	20	12-20	16	10***
	25	10-20	15	76***
	30	7-20	13	89***
	35	15-25	20	37***
<i>Citrus indica</i>	20	9-20	14	88***
	25	7-20	13	91***
	30	7-10	13	96***
	35	7-9	8	24.7***
LSD: the mean difference is significant at the 0.05 level (P < 0.001)				

Further, correlation analysis was also performed to obtain the optimum temperature required by the *Citrus* seeds to germinate. The regression equation derived from the second degree polynomial (parabolic curve) best describes the correlation of the effect of temperature on time for Citrus seed germination (Wiltbank *et al.*, 1995). The optimum temperature for germination was calculated from the derivative given by Rouse and Sherrod (1996).

$$\frac{dy^{diff}}{dx} = 2ax + b$$

$$0 = 2ax + b$$

$$x = \text{Max/Min or optimum temperature}$$

The equation was derived by subtracting the polynomial regression equation of the mean days to germination from percentage equation for each species.

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Table 2: Regression equations, correlation coefficient of temperature on days to germination and percentage germination of seeds of three <i>Citrus</i> species with significance level				
Species	Variable	Regression equation	R ²	Significance P value
<i>Citrus macroptera</i>	Mean days to germination	$0.075x^2 - 3.915x + 64.725$	0.844	0.000 (0.05)
	Percentage germination	$-1.180x^2 + 66.780x - 854.2$	0.8	0.000 (0.05)
<i>Citrus latipes</i>	Mean days to germination	$0.070x^2 - 3.570x + 58.5$	0.873	0.000 (0.05)
	Percentage germination	$-1.230x^2 + 69.430x - 884.950$	0.946	0.000 (0.05)
<i>Citrus indica</i>	Mean days to germination	$0.005x^2 - 0.765x + 28.255$	0.893	0.429 (0.05)
	Percentage germination	$-0.910x^2 + 47.270x - 512.550$	0.698	0.000 (0.05)

Table 2 and Figure 1 describe the polynomial regression equation of mean days to germination and percentage germination by temperature for all three species. Germination percentage and number of days required to germinate was positively correlated to the temperature. The germination increases with the increase in temperature from 20°C to 30 °C whereas with further increase to 35 °C, the germination percentage decreases.

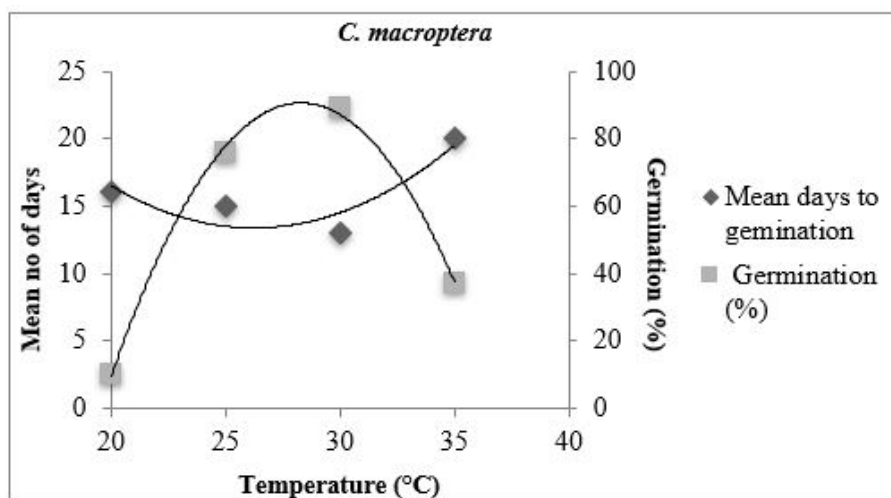


Figure 1(a): Mean days to germination and percentage germination in relation to temperature for *C. macroptera*

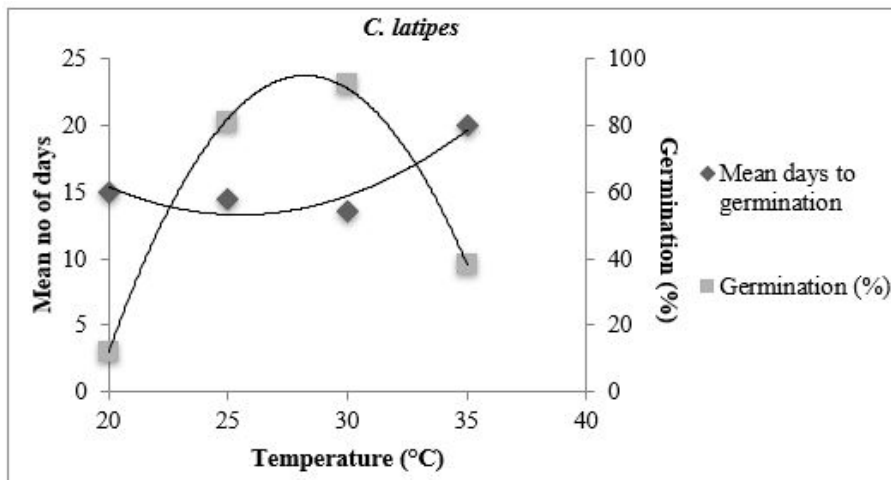


Figure 1(b): Mean days to germination and percentage germination in relation to temperature for *C. latipes*

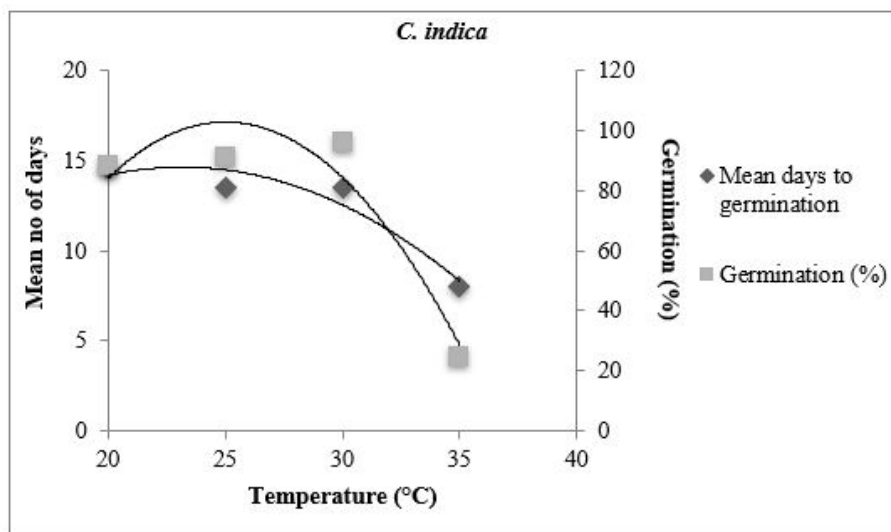


Figure 1(c): Mean days to germination and percentage germination in relation to temperature for *C. indica*.

The number of days required for germination decreased with the increase in temperature from 20 °C to 30 °C, but further increase in temperature to 35 °C, led to the reversing of effect leading to increase in the number of days required for germination except in *Citrus indica*. In *Citrus indica*, even after increase in temperature from 30 °C to 35 °C, time required for germination did not increase. The equation was positively correlated to temperature with the coefficient value of more than 0.8 in all cases except for *C. indica*.

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Table 3: Optimum Temperature, Germination Temperature Range and Days to Germination Range for the three <i>Citrus</i> species			
Species	Optimum Temperature (°C) for Germination	Germination Temperature Range (°C)	Days to Germination Range
<i>Citrus macroptera</i>	28.16	20-35	7-25
<i>Citrus latipes</i>	28.07	20-35	7-25
<i>Citrus indica</i>	26.24	20-35	7-20

Thus the relationship between the temperature and germination and time required based on regression analysis indicated that temperature enhances percentage germination and also time up to the optimum temperature beyond which it could be detrimental. Table 3 shows the calculated optimum temperature for germination for all three species. The graphical representation of the result also conforms to the obtained data. The optimum temperature for germination was near to 30°C. This point of optimum germination, defined as the temperature at which the greatest number of seeds germinated in the least number of days and also the overlap of the two curves are at the furthest distance from each other (Figure 1). Table 3 shows the optimum temperature for germination with the range of temperatures in which seed germination was carried out and range of days required for germination.

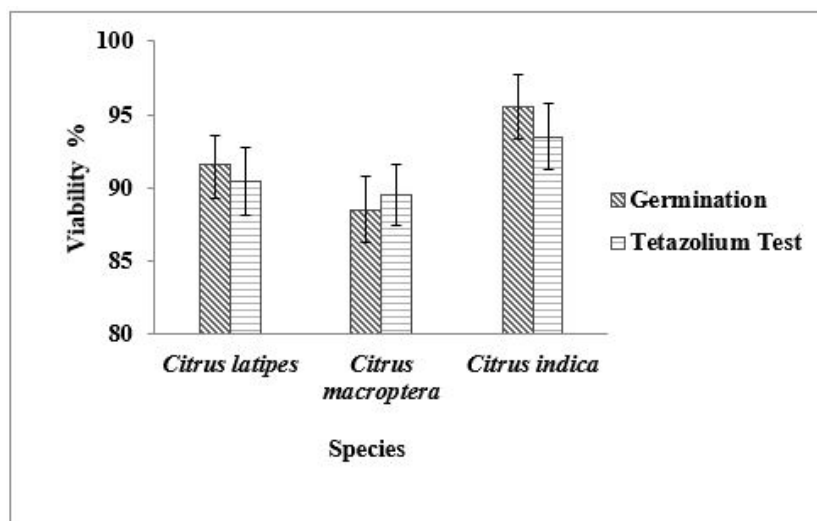


Figure 2: Percentage laboratory germination and viability of three *Citrus* species

Seed viability test of the three species of *Citrus* using TTZ test showed an average of 90 % viability in *C. macroptera*, 91 % in *C. latipes* and 94 % in *C. indica*. Seed viability tested using germination at the optimum temperature of 30°C for the three species showed the seed viability of 89 %, 92 % and 96 % respectively. Seed viability measured by tetrazolium staining, paralleled the germination percentage tested by seed

germination in the germinator at 30°C, the optimum temperature (Figure 2). ANOVA was used to test whether the two methods showed different results. It was found that the two tests did not differ significantly.

Discussion

Citrus seeds have been reported to germinate at a wide range of temperatures with the minimum temperature of about 6 °C and a maximum of about 39 °C and the optimum temperature for several varieties range from 26 °C to 30 °C (Camp 1933; Mobayben 1980; Soetisna *et al.*, 1985). In the present study, the optimum temperature for germination for the three species of *Citrus*, namely, *C. macroptera*, *C. latipes* and *C. indica* ranged between 26-28 °C. This result is in conformity with that of Ellis *et al.* (1985) and Rouse and Sherrod (1996), who also found similar temperature optima for *Citrus* seed germination.

Percentage germination increased with increase in temperature up to the optimum and subsequently it decreased with further increase in temperature above optimum, which is similar to the findings of Rouse and Sherrod (1996) in case of 17 varieties of *Citrus*. Detrimental effect of temperature higher than the optimum on germination of *Citrus* seeds has also been reported by Wiltbank *et al.* (1995). Mean days required for germination of seed of the three species of *Citrus* decreased as temperature increased up to the optimum. However, continued increase in the temperature above the optimum resulted in the increase in mean days required for germination of seeds of *C. macroptera* and *C. latipes*. Rouse and Sherrod (1996) and Ellis *et al.* (1985) also reported similar effect of temperature on germination of seeds of *Citrus* species. While in case of *C. indica*, with the increase in temperature from 30 to 35°C, the germination time decreased sharply but this resulted into very less total percentage germination as a large number of seeds were damaged at this temperature.

All the three species *viz.*, *C. macroptera*, *C. latipes* and *C. indica* grow in sub-tropical climate mostly on the hills at an elevation ranging from 500 m to 1900 m above msl. Temperature range suitable for seed germination of these species recorded in experiment are closer to ambient temperature of their places of occurrence in nature suggesting that probably temperature is one of the important ecological factor regulating the distribution of the *Citrus* species. The study has shown that sensitivity of three species to temperature varies. *C. indica* showed a high germination of 88% at a temperature as low as 20 °C, while in case of the other two species the seed germination at 20 °C was about 10%. The effect of temperature on seed germination of *C. latipes* and *C. macroptera* was found to be similar, whereas *C. indica* behaved in a different way. Bayer *et al.* (2009) based on their findings from cpDNA sequence study showed that *C. macroptera* and *C. latipes* are closely related while *C. indica* is genetically away from these two. Spiegel-Roy and Goldschmidt (1996) also classified *C. macroptera* and *C. latipes* in subgenera “Papeda” and *C. indica* in subgenera “*Citrus*”. These genetic variability may be the reason for the variation in their sensitivity to temperature.

Effect of temperature on germination of *Citrus macroptera*, *Citrus latipes* and *Citrus indica* seeds

Seed viability measured by tetrazolium staining, paralleled the germination percentage tested by seed germination in the germinator at 30 °C, **the optimum temperature. This indicates that almost all viable seeds of the *Citrus* species studied germinated at optimum temperature.** Similar findings were reported by Malik *et al.* (2006) on the viability of seeds of *C. macroptera* and *C. indica*. The findings of this study is also in close conformity with that of Saipari *et al.* (1998) who worked on the viability of seeds of *C. karna*, *C. jambhiri* and *C. grandis*. It was found that the optimum temperature for seed germination for the three species varied between 26 – 28 °C.

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Flavonoids and radiation response of cancer cells: A therapeutic prospective

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Abstract

Radiation therapy has become a critical part of cancer treatment. The fact that radiation induced damage affects both cancer and normal cells limit the effectiveness of radiation therapy. Radioprotectors and radiosensitizers can increase the effectiveness of radiation therapy. Flavonoids can act both as radioprotectors and radiosensitizers. As radioprotectors, flavonoids could up-regulate the anti-oxidant enzymes and the DNA-repair processes in the cells. As radiosensitizers, flavonoids inhibited the pro-survival NFkB, Akt pathways while up-regulating the apoptotic pathways. Cancer cells have a greater demand for metals to sustain their continuous proliferation and flavonoid-metal interaction is critical for the action of flavonoids to sensitize cancer cells to radiation.

Key words : Cancer cells, Flavonoids, Radiation, Response, Therapeutic

Introduction

Radiation therapy has become an integral part of cancer treatment procedures. However, radiation therapy has its serious issue of being a non-specific treatment process as radiation can't distinguish between the cancerous and healthy cells. Damage to the nearby healthy tissues can again have both immediate effects and late effects. Immediate effects stem from disruption in the fast dividing cells which would again lead to dermatitis, mucositis, hair loss etc. while the late effects includes tissue injuries leading to fibrosis, atrophy infertility, hormonal imbalances and tumour recurrence. There are also damages to the vascular tissues surrounding the organ receiving the radiation therapy leading to telangiectasia, bleeding etc (Barnett *et al.*, 2009). A common example of normal tissue damage during radiation therapy is radiation pneumonitis caused by damage to the lung tissues when the thoracic region is subjected to radiation therapy. The damage to lung tissues is a major dose limiting factor in radiation therapy of the thoracic region (Wang *et al.*, 2012). Keeping the issue of normal tissue toxicity in mind, radiation therapy doses are being moderated to gain maximum reductions in the tumours with minimal damage

to the healthy tissues. The strict maintenance of this balance between maximizing the therapeutic potential of radio-therapy while minimizing collateral damage to the adjoining healthy cells results in improved therapeutic ratio (Barnett et al., 2009)(Figure 1).

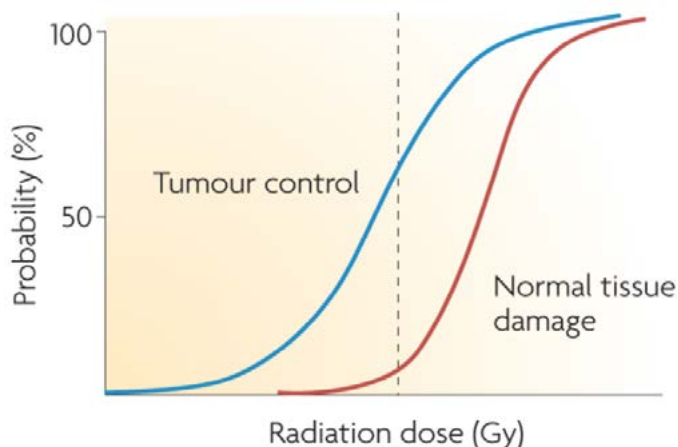


Figure 1: Dose response curve for radiotherapy showing the increasing probability of normal tissue damage along with increased tumour with an increase in radiation. The dotted line shows a theoretical dose to maximize tumour cytotoxicity with minimum damage to the normal tissues (Barnett *et al.*, 2009).

The dose response curve shows the difficulties in aiming for major tumour control with relatively fewer radiation therapies, which can be attributed to the necessity of preventing normal tissue damage. The limitation in the radiation dose that can be applied often leads to resistance of tumours in several cases, along with tumour recurrence. Both of these unfeasible events can be attributed to radiation-induced stress response in cancer cells. Repeated exposure to limited doses of radiation can promote the expression of several pro-survival factors like NFkB, Akt, MMP-2, cell cycle proteins and several pro-inflammatory cytokines, all of which contribute to increased resistance of cancer cells to radiation. The end result of these changes in gene expression in response to radiation therapy lead to lowered 2 year- and 5 year- survivality of cancer patients thus, lowering the overall efficacy of radiation therapy to treat cancers (Kim *et al.*, 2015).

The aim to improve the results of radiation therapy lead to the development of two different sets of compounds- radioprotectors and radiosensitizers. Radioprotectors protect the normal cells from radiation-induced damages thus, lowering side effects of radiation therapy while radiosensitizers sensitize the cancer cells to radiation leading to more radiation induced tumour cell death (Hazra *et al.*, 2011).

Radioprotectors

Radioprotectors are free radical scavengers which allow them to protect cells from free radicals that are produced in a living tissue upon exposure to radiation. Besides free radical scavenging, radioprotectors can act *via* inhibition of lipid peroxidation,

enhancement of DNA repair, stimulation of cell proliferation, immune stimulation, H-atom donation, and prevention of inflammatory reactions (Velpula *et al.*, 2013; Saaya *et al.*, 2017).

Several compounds have been tested as potential radioprotectors. These include-sulphydryl compounds like cysteine, amifostine; antioxidants like vitamin A, C, E; angiotensin converting enzyme inhibitors like pencillamine, captopril, immunomodulators such as gamma-interferon, polysaccharides AM5, and DNA binding ligands like Hoechst-33342 (Velpula *et al.*, 2013). Till date, only amifostine has been approved for use as a radioprotector of normal tissues in cancer radiotherapy (Patyar *et al.*, 2018). Amifostine prevented xerostomia upon head and neck irradiation while in case of chemoradiotherapy of NSCLC amifostine prevented pain and dysphagia, and also protected the normal cells from platinum induced cytotoxicity. The conversion of amifostine to its active form requires the action of alkaline phosphatase and the higher expression of alkaline phosphatase in the normal cells allows for the specific protection of the normal cells. However, amifostine has toxicity issues related to nausea, somnolence, vomiting and hypotension (Rosen *et al.*, 2015).

The safer alternatives of radioprotectors present plant extracts and plant polyphenols as natural choices. Several plants like *Withania somnifera*, *Curcuma longa*, *Eugenia jambolana*, *Tinospora cordifolia*, *Ocimum sanctum*, *Allium sativum*, *Emblica officinalis*, *Mentha piperita*, *Zingiber officinale*, etc. have shown radioprotective properties. The general method of radioprotection offered by the extracts derived from these plants consist of free radical scavenging, up-regulation of the expression of antioxidant enzymes like catalase, superoxide dismutase (SOD), glutathione transferase, glutathione peroxidase and DNA repair enzymes and down-regulation of PKC, MAPK, cytochrome P450 which are involved in mediating radiation induced damages (Bhandari, 2013).

Flavonoids have shown radioprotective properties. Flavonoids exerted their radioprotective effects *via* free radical scavenging, protection of the immune and haematopoietic system, anti-inflammatory mechanisms and protection of the DNA from damages (Li *et al.*, 2016). The ketone group in the flavonoid structure is important in preventing oxidative damage. Flavonoids also up-regulated the expression of antioxidant enzymes such as superoxide dismutase (SOD), glutathione transferase, glutathione peroxidase and DNA repair enzymes like DNA polymerase β , while down-regulating expression of genes like PKC, MAPK, cytochrome P450, NF κ B, COX-2 and LOX which are associated with promoting radiation related damages, inflammation and oxidative stress (Paul *et al.*, 2011; Jagetia *et al.*, 2012; Xu *et al.*, 2014). Flavonoids like silibin, apigenin, orientin, etc. are involved in protecting the DNA and repair of DNA in response to radiation (Satyamitra *et al.*, 2014; Li *et al.*, 2016). Almost all flavonoids show antioxidant activity with some flavonoids showing better radioprotection based on their better free radical scavenging properties. For example, genistein shows better free radical scavenging than quercetin and the combination of monoglycosylated rutin and quercetin shows better free radical scavenging and radioprotective properties as compared

to monoglycosylated rutin alone (Li *et al.*, 2016; Aizawa *et al.*, 2018). Flavonoids like apigenin, hesperidin and genistein stimulate the immune and haematopoietic system while baicalein prevents inflammatory reactions (Li *et al.*, 2016).

Radiosensitizers

Radiosensitizers have been used to get a higher cancer cell death at lower doses of radiation. Currently, the chemotherapy drugs are used as radiosensitizing drugs that are used in combination with radiation. The most common chemotherapeutic drugs used as radiosensitizers are cisplatin, 5-fluorouracil and taxanes and all of them have their own toxicity issues. Cisplatin is currently used as radiosensitizer in case of NSCLC (Alcorn *et al.*, 2013; Fong, 2016). Several other compounds are currently being tested as potential radiosensitizers. These include carbogen, a mixture of 95% oxygen and 5% carbondioxide; that leads to higher oxygen concentrations in cancer cells leading to greater free radical formation upon radiation exposure and thus, higher damage to the cells; camptothecin, a topoisomerase inhibitor; patupilone, a microtubule stabiliser; and monoclonal antibodies like ertolinib (Liman *et al.*, 2015).

Various plant extracts have also shown radiosensitizing properties, such as *Panax ginseng*, *Azadirachta indica*, *Tinospora cordifolia*, *Trametes versicolor*, *Withnia somnifera*, *Erythroxylum tuberosum* (Hazra *et al.*, 2012; Macedo *et al.*, 2016). Several flavonoids have been reported to have radiosensitizing properties and they include genistein, quercetin, flavopiridol, myricetin, apigenin and vicenin-2 (Garg *et al.*, 2005; Malik *et al.*, 2016; Prasad *et al.*, 2016; Baruah *et al.*, 2018). Flavonoids cause radiosensitizing effects on the cancer cells through various mechanisms. Radiation up-regulates PI3K/Akt, ERK, NFkB pathways which then set in motion several resistance mechanisms like up-regulation of anti-apoptotic proteins and DNA repair proteins (Abotaleb *et al.*, 2018). Flavonoids also inhibit the PI3K/Akt, ERK and NFkB pathway and promote apoptosis. In combination with radiation, flavonoids cause a greater reduction in cancer cell colony forming ability and also cause cell cycle arrest. Flavonoids exhibit a pro-oxidant effect leading to greater DNA damage. Flavonoids also cause down-regulation of the DNA repair pathways. Flavonoids promote apoptotic proteins and down-regulate the anti-apoptotic proteins. All of these factors contribute to the sensitization of cancer cells to radiation (Baruah *et al.*, 2018; Baruah *et al.*, 2019).

Flavonoids and metals and free radicals

The investigators group have checked the ability of three different flavonoids as radiosensitizers and also conducted test to check for their ability to ensure higher survival of embryonic HEK293T cells when exposed to radiation. The study included the flavonoids vicenin-2, naringenin and quercetin for their ability to sensitize lung cancer cells to radiation and all of them showed significant radiosensitizing ability. The specificity for flavonoid action could be attributed to the ability of flavonoids to undergo oxidation with metals resulting in the production of free radicals along with H₂O₂ which

leads to increase in flavonoid-induced apoptosis of cancer cells (Eghbaliferiz *et al.*, 2016; Uivarosi *et al.*, 2017). This complements the free radicals that are produced by radiation alone resulting in an excess of free radicals that the cancer cells have to put up with. An increased amount of free radicals was observed in the cancer cells when the cells were treated with vicenin-2 (Baruah *et al.*, 2019) and when the cancer cells were treated with the other two flavonoids (unpublished results). Cancer cells have higher levels of Cu ions due to increased requirement of Cu by the proteins involved in cell proliferation and angiogenesis (Wang *et al.*, 2010). The high Cu levels in turn allows higher levels of free radical and H_2O_2 production in the presence of flavonoids and thereby contributing to increased rate of apoptosis in the cancer cells (Eghbaliferiz *et al.*, 2016; Uivarosi *et al.*, 2017). At lower concentrations of flavonoid when the production of free radicals and H_2O_2 was less, flavonoids reported a cytoprotective activity on the cancer cells, which was observed in case of vicenin-2 (Sak, 2014; Baruah *et al.*, 2018), further highlighting the importance of free radical production by flavonoids to carry out their cytotoxic activities on cancer cells. The chelation of Cu by flavonoids also affects the Cu-dependent pro-cancerous processes and this area has now turned into a new focus point for researchers (Wang *et al.*, 2010). Normal cells have significantly lowered levels of metals as compared to cancer cells (Wang *et al.*, 2010) which could be taken as a primary indicator of an explanation regarding our observation of how the flavonoids we tested could offer radioprotection to the embryonic cells while being radiosensitive to the cancer cells. The flavonoids don't produce excessive free radicals in the embryonic cells that could affect the health of the cells.

Another aspect of the flavonoid-metal interaction has been the increase of lipophilicity of flavonoids when they are complexed with metals. Increased lipophilicity allows flavonoids to bind to the hydrophobic sites in the proteins, leading to clustering of the proteins to the complex, thereby affecting their functions. Flavonoids have also been reported to affect the membrane structure and the formation of lipid rafts which greatly affects the functioning of proteins like Akt and NFkB (Tarahovsky *et al.*, 2014). In our studies we have observed the lowering of the levels of activated Akt, which could be attributed to the disruption of lipid rafts. The lowering of activated Akt led to the lowering of the downstream anti-apoptotic proteins (Baruah *et al.*, 2019). However, we have studied on only a single cancer cell line and as such, the activities of the flavonoids need to be tested on more cancer models before establishing them as radiosensitisers and moving into clinical trials. The elucidation of the molecular mechanisms of the radioprotective effect of these flavonoids also remain fertile ground for future research.

Conclusion

Flavonoids can act both as radioprotectors and radiosensitizers and thus, have immense scope in being considered as regular clinical part of radiotherapy, thereby improving the effectiveness of radiation therapy without compromising the safety of the healthy cells and tissues adjacent to a tumour. The interaction of flavonoids and metals in both cancer cells and healthy cells, both with and without radiation, needs to be further

elucidated along with the molecular mechanism that these interactions might give rise to events on *in vivo* models.

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Nutritional status in relation with age and place of residence among the adult Rongmei Naga of Manipur

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Abstract

Nutritional status is a physical expression of the relationship between the nutrient intake, or bio-availability of nutrients and the physiological requirements of an individual. The present study is based on a cross-sectional sample of 678 Rongmei adult of Manipur aged 18-60 years. The paper deals with association between nutritional status, through Body Mass Index (BMI), and age and place of residence (rural vs. urban) among the adult Rongmei Naga of Manipur. BMI was calculated and later classified following Asian cut-off value of World Health Organisation (WHO). The result shows that 3.4% of the study population fall under underweight category, while 24.3% fall under overweight and 28.5% fall under obese categories. The mean BMI is found to be 23.52 ± 3.20 . The occurrence of overweight and obesity together are high which is to be considered as a matter of serious concern. It is observed that overweight and obesity are more prevalent among the urban Rongmei group than the rural Rongmei adult of Manipur.

Keywords: Rongmei Naga, Nutritional Status, Age group, Sex, Place of residence.

Introduction

Human health is a state of complete physical mental and social well-being, and not merely the absence of disease or infirmity (WHO, 1971). Adults are the major contributor to a population growth and development of any population, so study of adult health is very much in need of time and can be studied by looking into the health status. In order to alleviate the health status of adult, we should know the present conditions. Health is also closely interwoven with proper and adequate intake of food and nutrients. How well the body functions is a direct reflection of what we eat, how much we eat and the balance between the two. Thus the nutritional status of an individual is often the results of many inter-related factors, like food intake, quantity, ecological condition, socio-economic status and physical health (Wolanski and Malik, 1979). An adequate diet with the proper nutrients in the right amount as required by the body is considered to make an individual healthy while a poor diet characterised by intake of inadequate or excess nutrients in relation to the need can adversely affect the normal functioning of the body (National Institution of Nutrition, 2011). It is often the result of the complex interaction between

the food we eat, our overall state of health and the environment in which we live (WHO, 2000a). Therefore to understand the health status of any population, it is important to assess their nutritional status as it encompasses the whole and represents “the physical expression of the relationship between an individual’s dietary intake, the bioavailability of the ingested nutrients and his or her physiological requirements” (Brown, 1984), which in turn helps to identify individuals at risk of belonging to malnourished or who are malnourished.

Nutritional status in different population has been assessed by various methods involving the anthropometric variables of height and weight (Deurenberg *et.al.*1991). Anthropometry proves to be a useful tool in this nutritional assessment and is generally considered as the most universal applicable, inexpensive and non-invasive method available to assess the size proportions and composition of human body (Keys *et.al.*, 1956; WHO, 1986, 2011a; Gorstein and Arke, 1988; James *et.al.*, 1988; Frisancho, 1990; Shetty and James 1994; Prista *et.al.*, 2003; Ulijaszek and Komlos, 2010; Sanchez- Garcia *et.al.*, 2011). Anthropometry or the science of measurements of the human body has been the longest used measure of human variation (Ulijaszek and Komlos, 2010) and in hindsight it may be considered as the backbone of physical (biological) anthropology. From its initial application for the racial classifications in the nineteenth century to its use as a measure of physiological and developmental plasticity in adaptability researches from the twentieth century onwards, (Ulijaszek and Komlos, 2010), the use of anthropometry in anthropology has changed overtime.

In anthropometry, BMI study is widely accepted as one of the best indicator of the nutritional status of adult individual and population (Gogoi *et al.*, 2002). Adult health constitutes one of the serious problems affecting the community and the most common one is obesity. Obesity is a complex, multi-factorial, chronic disease involving, behaviour, perinatal and environmental components. It is a key risk factor for a range of chronic illness (including hypertension, diabetes, cholesterol, heart disease, stroke, gall bladder disease, biliary calculus, narcolepsy, osteoarthritis, asthma, apnoea, dyslipidaemia, gout and certain cancers) that tend to reduce the quality of life and ultimately result in death (Mora *et al.*, 2015). Rural areas in low and middle income countries have seen shifts towards higher incomes, better infrastructure and increased vehicles use, all of which bring health benefits. But this shift also led to lower energy expenditure and a hike in expenses on food, which is processed and low quality when sufficient regulations are not in place. Added to this, a significant number of obese patients tend to suffer mental disorders and social rejection leading to loss of self esteem, a particularly sensitive issue in the case of child. All these factors contributed to a faster increase in BMI in rural areas (Mora *et al.*, 2015; Centres for Disease Control and Prevention, 2017).

A number of studies have also found evidence that overweight and obesity are associated with increased prevalence of cardiovascular risk factors such as hypertension, unfavourable blood lipid concentrations and diabetes mellitus but does not appear to be the major risk factors for stroke (WHO, 2004). Keeping all these in mind, the present

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paper aims to understand the association of BMI with age and place of residence among the adult Rongmei Naga of Manipur.

Materials and methods

Rongmei, locally pronounce as N-ruang-mei, are indigenous people found inhabiting in the North East Indian states of Assam, Manipur and Nagaland. In Manipur, they are found concentrated in Tamenglong District, with a large number of villages in the valley districts of Imphal East, Imphal West, Thoubal and Bishnupur. "Racially the Rongmeis are one of the Southern Mongoloid decent and linguistically they belong to the Tibeto-Burman group of southern Mongoloid" (Pamei, 1996). They are also said to have migrated from China's Sinlung Province. Their physical features are by and large similar to the other tribes of North East India and people of the neighboring countries in South East Asia (Namthiubuiyang, 2001).

Height and weight were taken on 678 Rongmei adult individuals aged 18-60 from 338 households following door to door visit for collecting data. The 338 households were collectively from seven villages of Tamenglong district (rural) and Imphal East district (rural) of Manipur from both rural and urban places of residence. Weight was measured with minimal clothing with a standard weighing machine and height with Anthropometric rod. The subjects were asked to stand on the horizontal platform in the Frankfurt horizontal plane. Then the horizontal arm of the Anthropometer was brought down to touch the vertex lightly and the value was checked and taken. Ages of the individuals were taken by referring from their documents (Adhaar Card /EPIC/ Birth Certificate).

BMI (kg/m^2) was calculated following standard protocol ($\text{BMI} = \text{weight} / \text{height}^2$, kg/m^2). Age group was categorised as 18-28 years, 29-38 years, 39-48 years and 48years above till 60 years of age. Body Mass Index (BMI) was classified with Asia cut-off value as follows: Underweight = ≤ 18.5 , Normal weight = $18.5-22.9$, Over weight = $23-24.9$ and Obese = ≥ 25 (WHO 2000). BMI values were calculated with different age groups in two studied places of residence (rural and urban) of Manipur. Descriptive statistics were analysed by using MS excel software (Microsoft company 2007 version). Statistical analyses like t-test, chi – square test and regression model were analysed using IBM Developed software SPSS version 23. $P < 0.001$ is considered as significant for testing t-test and chi-square test in this paper.

Results

Table 1 shows the mean and standard deviation of the height, weight and BMI. Among all the adult Rongmei (male and female) interviewed, urban males mean height (158.22cm), weight (61.62kg) and BMI ($24.66\text{kg}/\text{m}^2$) are found to be the highest in both the places of residence. The rural females mean height (151.07cm) and weight (50.90kg) are found to be the lowest in the present studied population but BMI ($22.43\text{kg}/\text{m}^2$) is found to be lowest among the urban females. It is also observed from the results that the mean BMI, weight and height of the adult urban residents are higher than the adult rural

residents and the differences are statistically significant at 5% probability level (Table 1).

Table 1. Background characteristics of adult Rongmei Naga of Manipur

Parameters	Male		t-value	Female		t-value
	Rural	Urban		Rural	Urban	
	Mean±SD	Mean±SD		Mean±SD	Mean±SD	
Height	157.91±6.11	158.22±8.12	389.88*	151.07±5.94	152.92±5.56	503.24*
Weight	57.17±5.88	61.62±8.03	142.17*	50.90±6.89	57.16±8.54	125.08*
BMI	22.96±2.31	24.66±3.10	146.81*	24.16±3.77	22.43±2.86	129.70*

BMI= Body Mass Index; SD = Standard Deviation; *p-value < 0.001.

The BMI categories in relation to place of residence (rural and urban) and sex (male and female) are shown in Table 2.

Table 2. Nutritional status in relation with sex difference and place of residence among Rongmei Naga, Manipur

BMI Categories	Urban (N=314)				Rural (N=364)			
	Male		Female		Male		Female	
	N	%	N	%	N	%	N	%
Under weight	6	4.35	3	1.70	5	2.99	9	4.57
Normal weight	39	28.26	58	32.95	78	46.71	122	61.93
Over Weight	33	23.91	40	22.73	56	33.53	36	18.27
Obese	60	43.48	75	42.61	28	16.77	30	15.23

χ^2 value for urban vs rural male = 28.17* df=3 p<0.001

χ^2 value for urban vs rural female =44.21* df=3 p<0.001

Obese are relatively higher in urban males (43.48%) and urban females (42.61%) than their rural counterpart males (16.77%) and females (15.23%) respectively (Figure 1 and 2) From table 3, it is observed that, rural population are mostly with normal BMI (54.95%) out of 364 individual interviewed and in urban population normal BMI is found to be 30.89% out of 314 individual.

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Table 3. Descriptive statistics of nutritional status in relation with place of residence among the Rongmei Adult of Manipur

BMI categories	Urban (N= 314)		Rural (N=364)	
	N	%	N	%
Under weight	9	2.87	14	3.85
Normal	97	30.89	200	54.95
Over weight	73	23.25	92	25.27
Obese	135	42.99	58	15.93

$$\chi^2 = 66.39^*, df=3p<0.001$$

Here, obese (42.99%) are also found to be relatively higher among the urban residence comparing with the rural counterpart among the adult Rongmei of Manipur.

Table 4 shows the regression model of place of residence and age group on BMI in the Rongmei Naga of Manipur. Regression result suggests that ‘place of residence’ but not ‘age group’ has a significant effect on BMI in this population.

Table 4. Effect of place of residence and age on BMI on Rongmei adult of Manipur

Parameters	Coefficient		t-value	Significance level
	β	SD		
Age group	-0.014	0.030	-0.469	$p>0.05$
Place of Residence	-0.530	0.066	-7.975*	$p<0.001$

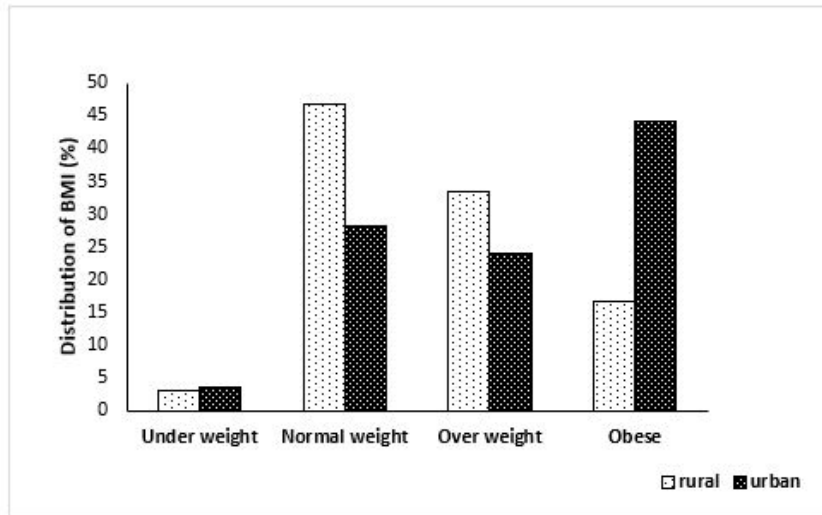
Dependent variable: BMI

It is also observed from the model that BMI is negatively influenced by age group, indicating the fact that with age there is tendency to decline in BMI, especially in elderly Rongmei. Similarly, the negative β coefficient of place of residence on BMI suggests that urban residents are significantly more susceptible to develop obesity as compared to their rural counterparts (Table 4).

Discussion

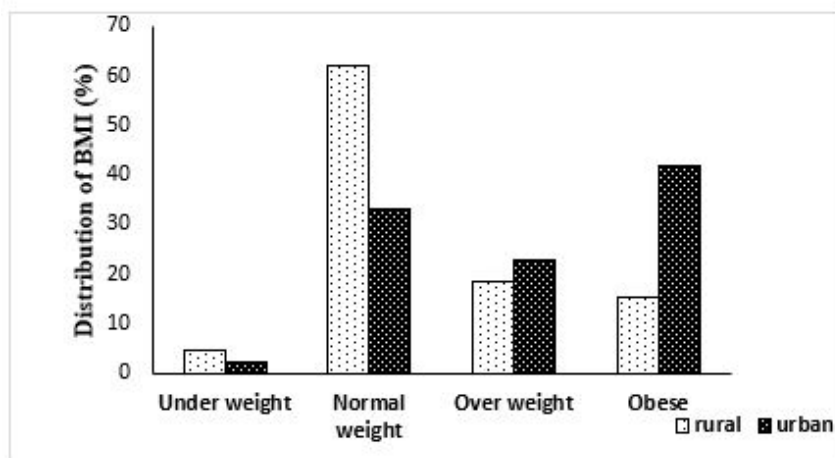
Among the Rengma Naga of Nagaland also, mean BMI, height and weight are higher among males (mean BMI = 22.66, mean height= 162.15cm and mean weight= 59.53kg), and lower among females (mean BMI = 22.30, mean height=156.08, mean weight= 54.37 (Seb Rengma et al. 2015), which is also the case with the present study.

Figure 1. Distribution of Nutritional Status according to the place of residence among the Rongmei male



Higher prevalence of obesity is also observed among the Rengma Naga of Nagaland (Seb *et al.*, 2015). In contrast to the present studied area (Manipur) researchers had come to conclusion that mean BMI are found to be higher in rural than in urban residence after an extensive study on 112 million people from 200 countries, including India over a period of 30 years. (Ezzati, 2016). Among the adult males of War Khasi of Meghalaya, age is negatively associated with weight and BMI (Khongsdier, 2002) which is the same among the present studied population.

Figure 2. Distribution of Nutritional Status according to the place of residence among the Rongmei female



Study by Mungreiphy among the Tangkhul males shows that the mean value (164.0 ± 6.16) was found to be highest in the youngest age group (20 to 29 years). Mean

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height decrease in each decade in the successive age group and lowest mean height (156.9 ± 5.35) was found among the oldest age group (60 to 70 years), whereas among the rest of the age group, the difference are statistically not significant. Among the Tangkhul males, the mean BMI is 22.3 kg/m^2 whereas among the Rongmei males of Manipur, the mean BMI is observed to be higher (23.7 kg/m^2).

Conclusion

In nutshell, greater percentages of obese individuals are found among the urban Rongmei than their rural counterparts, irrespective of gender. In other words, it is observed that nutritional status is highly significant with place of residence but not with age group among the Rongmei of Manipur. The reason could be sedentary life style with easily available calorie rich food in the urban areas in Manipur as compared to the rural areas. However, detail study on physical activity with dietary intake is required before concluding anything substantially.

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A proteomic approach to pesticide stress management in microalgae

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Abstract

Pesticides are one of the major sources of water pollution. Some pesticides are persistent organic pollutants and contribute to soil contamination. Microalgae are primary producers of ecosystem which are rich source of compounds having industrial values. Proteomic studies have highlighted the various mechanisms adapted by the microalgae in combating the pesticide stress. This review highlights the proteomics approach using Two-dimensional gel electrophoresis and MALDI-TOF for analysing the protein profile of microalgae.

Keywords: Pesticide, Microalgae, Two-dimensional gel electrophoresis, Mass Spectrometry

Introduction

Pesticides are used to control weeds, to repel or kill pests (insects, mites, nematodes etc.) in paddy fields, crops. They are used for controlling vector-borne diseases for health care of humans and animals. It includes insecticides, herbicides, fungicides, rodenticides, molluscicides etc. used to control pests (Damalas, 2009; Agrawal *et al.*, 2010; EPA 2012). Application of pesticides enhances the crop productivity, however under constant exposure to high concentration of chemicals, some pests have developed resistance to the pesticides which affects the non-target organisms such as microalgae and cyanobacteria and other aquatic organisms (Damalas, 2009). These pesticides enter the aquatic environment in different ways such as runoff, aerial drift and spill and affect non-target biota (Damala, 2009; Prado *et al.*, 2009). Pesticide drift occurs when pesticide particles suspended in the air are carried away by wind to other areas, potentially contaminating them. Pesticides heavily contribute to soil and sediment contamination (Lee *et al.*, 2011). In this way, pesticides enter into aquatic ecosystems from agricultural runoff or leaching and, as a consequence, have become some of the most frequent organic pollutants in aquatic ecosystems (Herrero *et al.*, 2009).

Pesticides are classified as (i) Organophosphates, (ii) Organochlorines, (iii) Carbamates and (iv) Pyrethroids. Organophosphate pesticides are the ester forms of phosphoric acid and are most widely used insecticide. Their main mechanism of action

is blocking the enzyme acetyl cholinesterase causing nervous and respiratory damages that result in the insect's death, but they are also hazardous to humans (Buchanan *et al.*, 2001). Some organophosphate pesticides are Chlorpyrifos, Malathion, Profenofos, Glyphosate, Quinalphos, Monocrotophos, Parathion, etc. The widespread use of these pesticides in agriculture has led to serious environmental pollution (Beard *et al.*, 2003). Organochlorine pesticides are derived from chlorinated hydrocarbons which alter the movement of ions across nerve membrane of the insects. They contribute in many acute nervous and chronic illnesses. Some chlorinated hydrocarbon pesticides are lipophilic i.e. they are easily accumulated and dissolve in fats and animal tissues and are not excreted, leading to adverse health effects in animals and human beings. Examples are DDT (Dichloro diphenyl trichloroethane) and PCBs (Polychlorobiphenyls) (Clary *et al.*, 2003; Rogan *et al.*, 2005). Carbamate are esters of carbamic acids. They are insecticide that work by inactivating the enzyme cholinesterase and alter the signal transduction in synapse. Example: Aldicarb, carbofuran, carbaryl, ethienocarb, fenobucarb, oxamyl and methomyl etc. Carbofuran is one of the most toxic carbamate pesticides. It is used to control insects in a wide variety of field crops, including potatoes, corn and soybeans. Pyrethroid pesticides are potent neuron poisons, endocrine disruptors and cause paralysis. Pyrethroids are synthetic version of pyrethrin a natural insecticide which is extracted from plant *Chrysanthemum*. They work by blocking the sodium ion channel in the insects. These are most commonly used insecticides introduced after organophosphates and organochlorine pesticides. Some examples are- deltamethrin, cypermethrin, alpha-cypermethrin (Dorman *et al.*, 1991; Damala, 2009).

Pesticides applied in the field are extremely detrimental to cyanobacteria (Agrawal *et al.*, 2013). Cyanobacteria (Blue green algae) are the largest and most widely distributed photosynthetic diazotrophs contributing to the carbon and nitrogen economy of paddy field soils and aquatic environment (Agrawal *et al.*, 2013). It has been reported that the interaction between *Anabaena* (cyanobacterium) and *Azolla* (water fern) have great importance in paddy fields, where nitrogen is frequently a limiting nutrient (Chellappa *et al.*, 2004). Some of the prominent rice field cyanobacteria are *Anabaena*, *Aulosira*, *Cylindrospermum*, *Gloeotrichia*, *Nostoc*, *Rivularia* and others (Venkataraman *et al.*, 1975). Green microalgae are photosynthetic eukaryotes whose primary function is to produce starch or carbohydrates. In spite of their simple physiology they can survive in harsh environmental conditions (Raven and Falkowski, 1999). The green microalgae and higher plants share metabolic pathways, which makes it susceptible to the action of herbicides (Lipok *et al.*, 2010; Tohge *et al.*, 2013). They are the primary producers in the aquatic environment and are rich source of compounds which have medicinal and industrial value (Borowitzka *et al.*, 2016). Pesticides which enter the aquatic environment has negative impact on these microalgae which have adverse effects on the whole aquatic ecosystem. Some microalgae are reported to accumulate and bioconcentrate, and biotransform xenobiotics. Microalgae are excellent biomonitors of pollution because of their sensitive nature to various contaminants. Studying the effects of these contaminants gives early warning of a polluting situation (Torres *et al.*, 2008). Different pollutants

induce oxidative stress in the microalgae. Some microalgae are able to tolerate such contaminants in a higher concentration. Omics approaches which involve genomics, proteomics, transcriptomics and metabolomics are applied to investigate the underlying principles of these organisms tolerating the contaminant. In this review we discuss about a proteomic approach in brief.

Proteomics Approach: Two Dimensional Gel Electrophoresis

Proteomics provides an effective way to identify and quantify proteome of an organism. It gives comprehensive insight into proteome changes in an organism upon stresses and reveals the mode of action, and identifies potential biomarkers (Tan *et al.*, 2012; Qiao *et al.*, 2012). Two dimensional gel electrophoresis coupled with mass spectrometry is an acclaimed tool for qualitative and quantitative assessment of proteomic changes (Zhang *et al.*, 2014). iTRAQ (isobaric tags for relative and absolute quantitation) is also a widely utilized quantitative proteomics approach to obtain relative quantification information of peptides in up to eight samples simultaneously. This method uses several isobaric tags to label peptides from various samples followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis to perform protein identification and quantitation. Since iTRAQ can quantify proteins from up to eight samples simultaneously, it's an appropriate method to investigate altered protein expression level from control and treated samples and determine the effect of exposure or treatment time on the organism (Tan *et al.*, 2012; Qiao *et al.*, 2012). Recently, alteration in the algal protein expressions induced by cypermethrin exposure has been studied by iTRAQ where photosynthetic proteins, stress responsive proteins and carbohydrate metabolism were found to alter (Gao *et al.*, 2016). However, two dimensional gel electrophoresis is more affordable and employed by many researchers than iTRAQ technique. Two dimensional gel electrophoresis coupled to mass spectrometry is an objective for the high quality separation of proteins. Proteins extracted from the control and pesticide stressed samples are subject to the 2-D gel electrophoresis. Two dimensional gel electrophoresis consist of first dimension i.e. isoelectric focussing and second dimension i.e. SDS-PAGE. In isoelectric focussing the isolated and purified protein sample is separated based on Isoelectric point in IPG strip in an isoelectric focussing chamber, and in second dimension this protein on the IPG strip is separated by SDS-PAGE where it gets separated based on their molecular mass. After this the gel is stained with Coomassie brilliant blue stain. Analysis of the gel image is carried out to check the differences in the spot intensity of both the control and treated protein samples. Following this, the differentially expressed protein spots are excised and in gel trypsin digestion is done. The digested peptides are further analysed by MALDI-TOF MS/MS. The results from the MALDI-TOF MS/MS analysis are further searched by MASCOT database software for the identification of the proteins.

Previous studies have reported the upregulation of various stress related proteins in microalgae such as oxidoreductase, photosynthesis related proteins, transporter proteins, carbohydrate metabolism, stress proteins and others to tolerate the stress (Gau *et al.*, 2016; Ismaiel *et al.*, 2018). Some cyanobacteria are reported to mineralize organophosphorus

compounds and use them as nutrient source. Phosphate specific transporters (Pst) and alkaline phosphatases were identified by proteomic approach to play an important role in utilization of these compounds as phosphate source (Tiwari *et al.*, 2014). Similarly, in another study *Nostoc muscorum* was found to be more tolerant to different concentrations of malathion than *Anabaena oryzae* and *Spirulina platensis* due to the presence of the enzymes which can hydrolyse and utilize this organophosphorus compound as nutrient source (Ibrahim *et al.*, 2014). Genes encoding a number of organic pollutant degrading enzymes are also reported. The gene *cbaA* that encodes enzyme 3-chlorobenzoate-3, 4-dioxygenase is upregulated by 1.26 to 8.9- fold during PCB (Polychlorobiphenyl) degradation by *Anabaena* PD-1. These proteins were identified using Two dimensional gel electrophoresis (2-DE) coupled with matrix-assisted laser desorption/ ionization time of flight mass spectrometry (MALDI-TOF MS). These findings reveal the resistance and adaptation of cyanobacterium to the presence of PCBs (Zhang *et al.*, 2014). Some cyanobacteria and green algae are reported to degrade pesticides into less toxic metabolites. As example, *Spirulina platensis* is found capable of degrading chlorpyrifos to its less toxic primary metabolite 3,5,6-trichloro-2-pyridinol (TCP) in laboratory cultures by HPLC analysis (Thengodkar *et al.*, 2010). Green algae such as *Scenedesmus* sp. MM1, *Scenedesmus* sp. MM2, *Chlamydomonas* sp., *Chlorella* sp., *Stichococcus* sp., and five cyanobacteria *i.e.* *Nostoc* sp. MM1, *Nostoc* sp. MM2, *Nostoc* sp. MM3, *Nostoc muscorum* and *Anabaena* sp. were reported to biodegrade organophosphorus pesticide fenamiphos into fenamiphos sulfoxide (FSO), which is further hydrolyzed to fenamiphos sulfoxide phenol (FSOP) analysed by using HPLC (Caceres *et al.*, 2008). *Synechocystis* sp. strain PUPCCC 64 can tolerate chlorpyrifos pesticide up to 15 mg/L. GC-MS analysis showed this organism can degrade chlorpyrifos into less toxic products as 3,5,6-trichloro-2-pyridinol (TCP) (Singh *et al.*, 2011). Studying these organisms by proteomic approach will further reveal the proteins involved in the various degrading pathways. Specific gene characterized from tolerant species of cyanobacteria was further explored for its potential by cloning in other bacteria. As reported, in *Anabaena* sp. PCC7120 phytochelatin synthase (*pcs*) is involved in the synthesis of phytochelatin (PCs) which plays role in heavy metal detoxification. The effect of an extensively used rice field herbicide butachlor was also studied on three *Anabaena* species *e.g.* *Anabaena* sp. PCC 7120, *Anabaena doliolum* and *Anabaena* L31. 75 differentially expressed proteins from each *Anabaena* sp. were reported which are related to photosynthesis, carbon, nitrogen and protein metabolism, redox homeostasis, and signal transduction. Early accumulated proteins involved in photosynthesis (*atpA*, *atpB*), carbon metabolism (*glpx*, *fba* and *prk*), protein folding (*groEL*, *PPIase*), regulation (*orrA*) and late accumulated proteins are involved in *Anabaena* L31 and in *Anabaena* sp. PCC 7120 to tolerate prolonged exposure to butachlor (Agrawal *et al.*, 2014).

Recent study also reported the tolerance strategy of cyanobacterium *Fischerella* sp. under methyl parathion (MP) stress which is investigated through proteomics analysis using 2-DE technique coupled with MALDI- TOF MS/MS. This cyanobacterium treated with MP for 2 and 8 days showed differential expressions of proteins related to

photosynthesis, energy and protein metabolism, redox homeostasis, signal transduction and cellular defence compared to the control (Tiwari *et al.*, 2018).

Conclusion

Proteomics approach has highlighted the comprehensive view of various mechanisms adapted by the microalgae in combating the pesticide stress. These microalgae further are a rich source of various compounds which have industrial value. So studying the microalgae in their proteome level will help to identify stress responsive proteins which can further be characterised as potential biomarker for the future investigation of pesticide exposed plants and microalgae.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Isolation and identification of cellulose degrading bacteria from gut of two herbivorous pest larvae

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Abstract

*Insect gut microflora contributes towards the utilization of complex food resources in the gut of the host. The interaction of the host and the micro organisms can be symbiotic or transient. The present study explored the gut flora of two Lepidopteran pest larvae namely black cutworm (*Agrotis ypsilon*) and *Colocasia esculenta* leaf roller (*Cnaphalocrocis* sp.) to isolate potential cellulolytic bacteria from their gut. We could screen a total of 19 cellulolytic bacterial isolates from the gut of these two insect species. The bacterial isolates were aseptically isolated and screened on CMC (Carboxymethylcellulose)-agar medium by using CMC as a sole carbon source. Their cellulolytic potential was checked by using Congo-red overlay method and Gram's iodine method. The latter method was found to be more efficient with rapid and distinctly visible zone of hydrolysis. Basic identification of the bacterial genera was conventionally done by Bergey's Manual of Systematic Bacteriology based on their biochemical properties. Their cellulolytic efficiency was determined based on CMCase and FPase (Filter paper) assays. Estimation of reducing sugar was done as per DNSA (3, 5- Dinitrosalicylic acid) method (Miller, 1959).*

Key words: *Agrotis ypsilon*, *Cnaphalocrocis* sp., Lepidoptera, Congo-red, Gram's Iodine, Carboxymethylcellulose, CMCase, FPase assay, DNSA.

Introduction

The cellulolytic capacity of microorganisms is of great importance from the stand point of industrial microbiology and in terms of microbial ecology. To determine the number of cellulolytic microorganisms present in any complex ecosystem pose great challenge (Teather and Wood, 1982). There are two possible ways for enumeration of cellulolytic bacteria namely direct and indirect method. The direct enumeration method seriously underestimates the number of cellulolytic bacteria present in the studied environment (Bryant and Burkey, 1953). Whereas, in case of indirect enumeration

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method, by using a non-selective medium to isolate wide range of bacteria, followed by testing of each isolate for cellulolytic activity, severely limits the nature and number of studies undertaken (Smith, 1977; Mahasneh and Stewart, 1980). So an alternative method was proposed to use a soluble substrate (carboxymethyl cellulose) which gets precipitated with detergent to visualize a zone of hydrolysis on an agar plate (Hankin and Anagnostakis, 1977). The interaction of the direct dye Congo-red with intact β -D-glucans provides the basis for a rapid and sensitive assay system for bacterial strains possessing β -(1 \rightarrow 4),(1 \rightarrow 3)-D-glucanohydrolase, β -(1 \rightarrow 4)-D-glucanohydrolase and β -(1 \rightarrow 3)-D-glucanohydrolase activities (Teather and Wood, 1982). Congo-red overlay method is considered to be most popular staining technique for selective technique. But it has been reported that staining with Congo-red has less efficiency and it also deactivates the microbes. Hence, Gohel *et al.*, 2014 proposed that Gram's iodine solution as an alternative stain for the determination of extracellular activity on CMC agar plate.

A large number of bacteria are capable of degrading cellulose, but only a few of them produce significant quantities of cell-free bioactive compounds capable of completely hydrolyzing crystalline cellulose *in-vitro* (Patagundi *et al.*, 2014). There is a rise in demand for stable, highly active and specific cellulase enzyme for industrial application (Cherry and Fidants, 2003). The degradation of cellulosic materials has been reported from numerous studies, but few reports are there regarding which organisms met the industrial requirement (Lee *et al.*, 2008). It also have been reported that bacteria with higher growth rate as compared to fungi, also considered having a better potential for cellulase production (Sethi *et al.*, 2013). So it is utmost necessary to keep on exploring new sources of cellulase enzymes. Insect gut micro-flora contributes towards the utilization of complex food resources in the gut of the host. Herbivorous insects harbour pool of microorganisms which aid in their digestion. Our present investigation was designed to isolate and screen cellulolytic bacteria from two Lepidopteran pest larvae gut.

Materials and methods

Isolation of bacteria

The insect larvae were collected freshly from field in and around the North-Eastern Hill University (NEHU) campus. The larvae were killed by putting in deep freeze for half an hour and were surface sterilized with 5.25% sodium hypochlorite solution prior to dissection. The dissection was done aseptically under laminar air flow hood. The microbes present in the guts were enumerated by serial dilution of macerated gut content in distilled water onto CMC agar plate as inoculum. The media composition (g L⁻¹) for isolation of cellulolytic bacteria contains: 10g peptone, 10g CMC, 0.2g MgSO₄, 0.5g NaCl₂, 0.1g CaCl₂, 15g Agar at pH 6.5. CMC was used as a sole carbon source. The inoculated plates are incubated at 32°C for 24-48 hours (Dantur *et al.*, 2015).

Screening of bacteria

The incubated CMC agar plates were flooded with 1% Congo-red solution and

allowed to stand for 15 minutes. The dye was poured off and the plates were washed with 1M NaCl₂ thoroughly several times. Bacterial isolates producing cellulase enzymes produced clear zone of hydrolysis around the colony. We flooded the duplicate CMC-agar plate with Gram's Iodine solution to check the cellulolytic potential of the isolates. The bacterial colonies having clear zones were selected for further analysis. Further bacterial stains were purified by repeated streaking. The pure cultures were preserved at 4°C till further investigation.

Screening for cellulase enzymes

Development of inoculum

The selected bacterial isolates were inoculated in broth medium (gL⁻¹) containing: 1.5g KH₂PO₄, 2.5g Na₂HPO₄·7H₂O, 0.3g MgSO₄·7H₂O, 0.5g NaCl₂, 0.1g CaCl₂, 0.005g FeSO₄·H₂O, 0.0016g MnSO₄, 10g CMC at pH 6.4. The broth cultures were incubated in shaker incubator at 37° C up to 120 hours at 150 rpm. At intervals of every 24 hours the bacterial cultures were collected in 15 ml centrifuge tube and centrifuged at 12000 rpm for 10 minutes in cooling centrifuge to collect supernatant. The supernatant obtained after centrifugation served as crude enzyme source.

Estimation of cellulase enzymes

Estimation of cellulase enzyme activity was assayed using 3, 5 – Dinitrosalicylic acid (DNS) reagent (Miller, 1959) by estimation of reducing sugars released from CMC (CMCase assay) and Whatman no. 1 filter paper (FPase assay). For CMCase assay 2% Carboxymethylcellulose (w/v) (2g CMC in 0.5 M sodium citrate buffer pH 5.5) and for FPase assay 50 mg Whatman no. 1 filter paper (1.0 cm × 6.0 cm) strips saturated in 0.5 M citrate buffer pH 5.5 were used. To the test tubes 2% CMC (w/v) with 250 µl of crude enzymes were added and incubated at 50° C for 30 min. For FPase assay filter paper strips and 500 µl of crude enzyme was added to test tubes and incubated for 60 min at 50° C. To the incubated mixture 3ml DNS reagent is added to stop reaction and heated in boiling water for 5 min to develop colour. The test tubes are allowed to cool down and 1 ml of Rochelle salt are added to each tube when they are still warm. Reducing sugar liberated during the reactions was measured as absorbance at 540 nm. One unit (IU) of enzyme activity was defined as the amount of enzyme that released 1 µmol of reducing sugar equivalent to glucose (mg min⁻¹) mg⁻¹ protein during the reaction (Ghose, 1987)

Identification of bacterial isolates

Bacterial isolates were identified by using standard identification tests as per Bergey's Manual of Determinative Bacteriology (Buchnan and Gibbons, 1974).

Optimization of culture condition

Optimization of temperature- To determine the effective temperature for cellulase production by the bacterial strains, isolates were incubated at different temperature viz.

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25⁰ C, 37⁰ C, 45⁰ C and 60⁰ C.

Optimization of pH- The most suitable pH for the cellulase production was determined by adjusting the pH of the medium to 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0.

Optimization of incubation period- Some microorganisms produce maximally during their exponential phase, whereas others in their stationary growth phase. The incubation period ranged from 24 hours to 120 hours, the assay was performed at an interval of 24 hours.

Optimization of salinity- Optimum salinity is required to maintain osmolarity of cell cytoplasm. Therefore, percentage of salinity ranging from 1-6 was investigated.

Morphological and biochemical characterization

The isolates showing highest cellulolytic activity were further subjected to morphological characterization, oxidase test, catalase test, MR VP test, citrate test, TSI test, Indole test, Urea hydrolysis test, Nitrate reduction test, Starch hydrolysis test, DNase test, Carbohydrate fermentation tests. The results of morphological characterization and biochemical characterization are summarized in the Table 1 and Table 2 respectively.

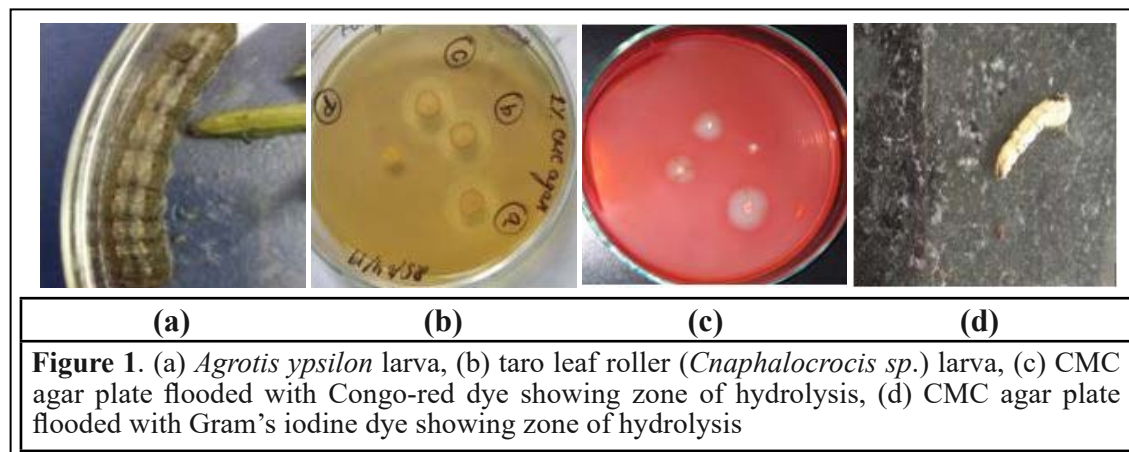
Results and discussion

A total of 19 bacterial isolates were screened from two Lepidopteran insects, 12 isolates from taro leaf roller (*Cnaphalocrocis sp.*) larva gut and 7 isolates from gut of *Agrotis ypsilon* larva. All the isolates showed positive cellulolytic activity on CMC agar plate when stained with both Congo-red and Gram's iodine solution. But later when cellulase activity was examined only two isolates (CWI 6 and TCI 11) one each from two insect larvae showed considerable cellulase activity (both CMCase and FPase). The result revealed that both isolates showed higher FPase activity than CMCase activity. Previous study reports very low CMCase activity in Lepidopteran gut fluid (generally < 0.06 U/mg proteins). The origin of cellulolytic enzymes probably reflects the phylogenetic relationship and feeding strategies of different insects (Su *et al.*, 2013). Morphological and physiological characteristics intensely depend on environmental conditions, hence the accurate identification of isolates turned out to be very difficult (Bakri *et al.*, 2010). The molecular techniques are more significant for the characterization of the new isolates, allowing grouping the strains.

Table1. Morphological characterization of bacterial isolates

Colony Morphology	TCI 11	CWI 6
Size	5 mm	1.3 cm
Colour	Creamy white	Creamy white
Elevation	Flat	Flat
Margin	Irregular	Irregular
Opacity	Opaque	opaque
Gram's staining	+ve (rod)	-ve (rod)
Motility	+ve	-ve

Furthermore, complex studies (morphological, biochemical and molecular) are essential, when the identification of new isolate is the purpose of the investigation (Rathnan *et al.*, 2013). Enzyme production is closely controlled in microorganisms and for improving its productivity, these controls can be improved. Cellulase yields appear to depend on a complex relationship involving a variety of factors like inoculums size, pH value, temperature, and presence of inducers, aeration, growth time, and so forth (Immanuel *et al.*, 2006).



During the present investigation it was found that the zone of hydrolysis was more prominent and readily visible when the CMC agar plate was stained with Gram's iodine (GI) than conventional staining method with Congo-red (CR) (Figure 1). The diameter of zones of hydrolysis was found to be 2.1 cm and 1.5 cm respectively for Gram's iodine and Congo-red staining method showed by CWI 6 bacterial isolate of a colony with diameter of 1.3 cm. For TCI 11, a colony with a diameter of 5 mm gave zone of hydrolysis of 1.2 cm and 7 mm for GI and CR respectively. So, the cellulolytic index was found to be higher with Gram's iodine than Congo-red staining technique (Table 3). Clear zone appeared around growing bacterial colonies indicating cellulose hydrolysis (Irfan *et al.*, 2012).

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Cellulolytic potential of the cellulose hydrolyzing bacterial isolates was determined by calculation of their Hydrolysis Capacity (HC) (Lloyd and Tarun, 2016). Cellulolytic index was calculated by using formula as follows (Ferbiyanto *et al.*, 2015):

$$\text{Cellulolytic index} = \frac{\text{Diameter of zone} - \text{Diameter of bacterial colony}}{\text{Diameter of bacterial colony}}$$

Bacterial isolate CWI 6 from *Agrotis ypsilon* insect gut showed maximum activity in its lag phase at 120 hour culture.

Table 2.Biochemical Characterization of the bacterial isolates

Biochemical properties	TCI 11	CWI 6
Catalase	+	+
Oxidase	-	-
MR-VP	++	++
Indole	+	-
Citrate	+	-
TSI	Fermentation(-),gas (-), H ₂ S (-)	Fermentation(+),gas (-), H ₂ S (-)
DNase	+	-
Nitrate reduction	+	+
Carbohydrate utilization		
(a) Mannitol	+	+(gas)
(b) Inositol	+(gas)	+
(c) Sorbitol	+(gas)	+
(d) Maltose	+	+
(e) Galactose	+	+(gas)
(f) Lactose	+	+(gas)
(g) Sucrose	+(gas)	+

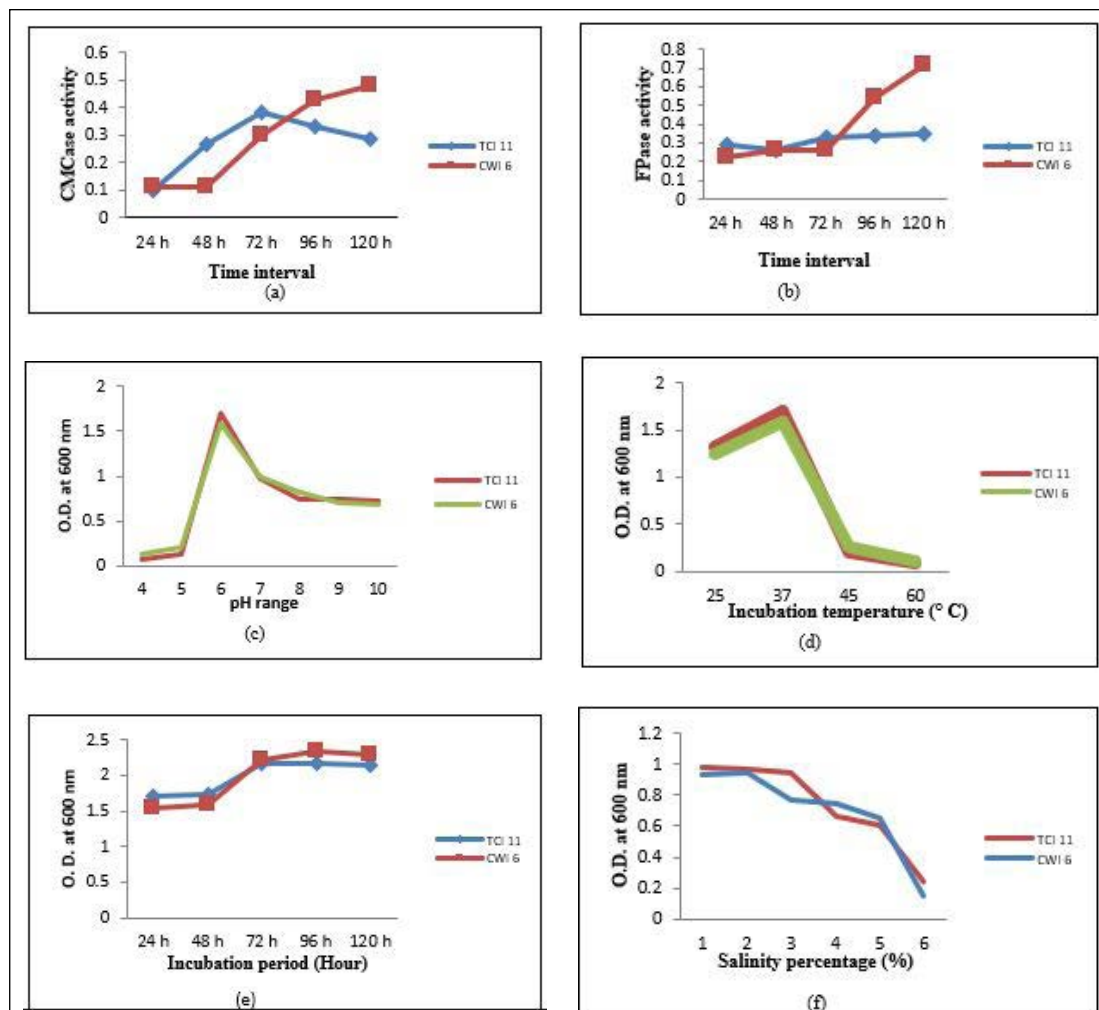


Figure 2 (a). CMCase activity of two bacterial isolates TCI 11 and CWI 6 at different time intervals. (b) FPase activity of two bacterial isolates TCI 11 and CWI 6 at different time intervals. (c) Effect of pH on bacterial growth. (d) Effect of temperature on bacterial growth. (e) Bacterial growth curve. (f) Effect of percentage of salinity on bacterial growth.

Whereas, TCI 11 from taro leaf roller larva gut showed highest CMCase activity at 72 hours incubation period and there was a sharp decline in its activity. But it showed maximum FPase activity at 120 hours incubation (Figure 2a and b). The optimal growth condition of the bacterial isolates revealed that both of them are mesophilic, neutrophilic, and can withstand up to 2% of salt concentration (Figure 2c, d and f).

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Table 3.Cellulase assay and cellulolytic index

Isolates	CMCase assay	FPase assay	Cellulolytic index (GI)	Cellulolytic index (CR)
TCI 11	0.233 IU	0.279 IU	1.4	0.4
CWI 6	0.378 IU	0.508 IU	0.615	0.153

Conclusion

With the help of biochemical tests (summarized in Table 2) we could conclude that bacterial isolate CWI 6 belongs to genus *Klebsiella* and TCI 11 belongs to genus *Bacillus*. The isolate CWI6 belonging to phylum Proteobacteria and TCI 11 belongs to phylum Firmicutes. Both families are reported by Huang *et al.*, 2012 to harbour a pool of cellulolytic bacteria. In contrast to the previous reports which claims that the CMCase activity in lepidopteran gut fluid is less than 0.06 U/ mg protein we could get better cellulase activity in case of both isolates from two different insect larvae gut.

Acknowledgement

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Agro-forestry: An alternative for Jhum Cultivation in Meghalaya

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Abstract

Jhum cultivation is considered highly destructive to environment as it involves burning and clearing vast areas of forest so that cultivation can be done for a few years. It does not only destroy forest but has a chain of effects and after-effects leading to multifarious adverse conditions of soil and climate. People like to cling to this method because it is simple and cheapest method, and also their ancestors practiced it with a sense of socio-religious importance. Recognizing the adverse effects of jhum cultivation the State Government, through the Soil and Water Conservation Department, took up jhum control programme since 1974-75 under the State Plan, but it has not achieved encouraging results. Eminent scientists have recently advocated Agro-forestry as an alternative to jhum cultivation. This article attempts at giving some details about the proposed method of Agro-forestry)

Key words: Cropping, Dibbling, Erosion, Denudation, Depredation

Introduction

Jhum cultivation is one of the most ancient systems of farming believed to have originated in the Neolithic period around 7000 BC. It is called slash and burn method of cultivation. The system is regarded as the first step in transition from food gathering and hunting to crop husbandry. It is still practiced in Khasi Hills and in the hill areas of the North East states including certain pockets of India. Among the Khasis, this system of cultivation is known as “*Thang shyrti*” or “*Thang bun*”. (Shangpliang, 2010)

The operation of jhum cultivation is started by jungle cutting in the months of December to January. After the jungle is cut down and cleared the debris is left to dry in the open. From the month of February to March setting of fire to the dried debris is carried out. This operation is done with care so as to avoid forest fires from spreading across to other hills, not meant for ‘*jhuming*’.

Sowing and planting of crops is done by dibbling in an intimate mixture of varied crops. Upland paddy is the main crop grown in mixture with maize, millet, sorghum, tapioca, chillies, cotton, turmeric, pumpkin, etc. Cropping is done with minimum tillage: No animals or large implements are used for preparing land. The only implements used

are the chopping knife, sickle, dibbling stick, spade and hoe. After the harvest the land is abandoned for 3 to 5 years to rejuvenate itself and jhuming is shifted to another site and repeated the same process.

Jhum cultivation is considered destructive to the environment, as vast areas of forest is cleared and burnt down so that cultivation can be carried out at least for 3 to 5 consecutive years. It does not only destroy forest but also disturb the ecological balance and destroys the environment. It has a chain of effects and after-effects leading to multifarious adverse conditions of soil and climate. An assessment made by TREES-II in one of its International initiatives of shifting cultivation practices in North East India revealed that in many states due to reduction in the fallow period from 20-30 years in the past to as low as 3 years in recent times the situation has attracted the attention of planners and decision makers. The re-growth of forests was almost nil and existing forests were also decreasing, the threat to which the forests are being subjected to is of immediate concern. (Roy *et al.* 2002) The following adverse effects of jhum cultivation show how the system causes far-reaching disturbances in the conditions of the soil leading to changes in the climate, ecological imbalance and the environmental degradation, besides fertility loss and low productivity of crops.

Adverse effects of Jhum cultivation

- a) Denudation of forests : Ecological Imbalance
Elimination of source of water
- b) Soil Erosion : Stream silting : Floods
Loss of soil fertility: Pressure on land
Low productivity
- c) Low Technology : Labour Intensive
Reservoir : Derogatory Development
No subsidiary Income
- d) Social Effects : Social custom : Large family size

The first adverse effect of jhum cultivation is denudation of forests leading to two consequences, viz. ecological imbalance and elimination of the sources of water and ecological imbalance. In fact the forest eco-systems of North East India as a whole is under severe pressure taking a heavy toll on both biotic and abiotic sources, due to population explosion, encroachments on forest lands, loss of forest cover for other non-forest users, shifting cultivation practices and degradation caused by illicit felling, logging for fuelwood forest and fodder etc. (Toky.1981) A recent study conducted by the ISFR (India State of Forest report, 2017) shows a net decline of 116 sq.km in the state of Meghalaya due to rotational felling and developmental activities whereas a slight increase has been due to plantation activities (Table 1).

Table 1 : Forest cover of North Eastern States

STATE	Geographical Area (sq.km)	VDF	MDF	% of	Total	Change
Arunachal Pradesh	83,743	20,721	30,955	22.83	66,964	-190
Assam	78,438	2,797	10,192	53.78	28,105	567
Manipur	22,327	908	6,510	57.24	17,346	263
Meghalaya	22,429	453	9,386	42.62	17,146	-116
Mizoram	21,081	131	5,861	67.05	18,186	-531
Nagaland	16,579	1,279	4,587	53.03	12,489	-450
Sikkim	7,096	1,081	1,575	20.57	3,344	-9
Tripura	10,486	656	5,246	23.61	7,726	-164
Grand Total	2,62,179	28,026	74,312	40.26	1,71,306	-630

Source : ISFR Report of 2017

The second adverse effect of jhum cultivation is that it causes soil erosion that affects fertility of the soil resulting in low productivity and pressure on land. According to a study conducted by the ICAR (Jha and Sarma, 2008) the average soil loss due to shifting cultivation in Meghalaya has been found to be 40.9 tonnes/ha. The highest sediment yield, however was, 76.9 tonnes/ha/year. The annual loss of soil was computed by taking into account an average soil loss of 40.0 tonnes/ha/yr. The report also indicated that annual soil loss due to shifting cultivation in the region was found to be about 15.5 million tones. A study conducted by Jha and Rathore (1981) revealed that erosion ratio of Orissa soil under shifting cultivation were 18.03 and 18.78 in the surface and 6.5 and 11.4 were in the sub-surface as compared to erosion ratio of soils under shifting cultivation of 10.7 and 10.8 in the surface and 3.7 and 11.7 in sub-surface soils. They also found that the erosion ratios were higher in the upper layer than in the lower layers. Another study conducted by Borthakur *et al.* (1978) revealed that the surface runoff under shifting cultivation was about 11.40 mm partial terracing reduced surface runoff further to 32.8 mm, indicating thereby that, physical barriers considerably reduces the surface run off.

It has been estimated that the average soil loss from the first year and second year of *jhumming* is 146.6 and 170.2 tonnes per hectare per year respectively. This clearly indicates that the second year of *jhumming* is comparatively more hazardous than the first year.

Thirdly, It causes rapid loss of soil nutrient. Together with the loss of valuable

fertile topsoil there is also corresponding loss of nutrients available in the soil. A study made on the affect of jhum on loss of soil nutrient conducted by Mishra and Ramakrishnan (1983) at high elevation of Meghalaya using 15, 10 and 5 years jhum cycle and terrace system showed that the soil nitrogen concentration under a five year cycle was significantly lower than under the 10 and 15 year cycles. The concentration of this nutrient declined sharply after the burn in the surface layers and was attributed to volatilization. The degree of volatilization is dependent on the intensity of the burn and, therefore, the nitrogen decline is lower in a five year cycle than in longer cycles. The variance in heat intensity during the time of jhum burning causes high or low decrease in the soil organic matter. Destruction of organic content is the highest when the intensity of heat above 150 degree Centigrade.

Present Status of Jhum Cultivation

The estimated *jhumia* population dependent on *jhum* is 13.87 percent of the total rural population of 18.53 lakhs of the state of Meghalaya in the year 2001 (Statistical Handbook Meghalaya, 2017). This clearly indicate that the population dependent on *jhum* had declined by 27.04 percent in a period of thirty years time that is from 40.91 percent out of the total rural population of 8.65 of the state in the year 1971. It has also been observed that the area under jhum cultivation in Meghalaya has been declining gradually .The following table shows the decadal change in the extent of shifting cultivation in Meghalaya from 2000 to 2010 (Table 2).

Table 2 : Decadal change in the extent of shifting cultivation in Meghalaya(2000-2010)

State	Shifting Cultivation Area (2000)	Shifting Cultivation Area (2010)	Change (Km)	% of Decadal Change
Meghalaya	2086.77	448.99	-1637.78	-78.48

Source : Indian Council of Forestry Research and Education and published under Statistical Year Book -2014 by MoSPI

At present with rapid increase in human population, there is corresponding heavy pressure on land. The *jhum* cycle has reduced drastically over the last few decades to an average of below ten years and in extreme cases such as that in Garo hills to as short as 3 to 5 years. In due course of time this cycle may even be brought down further. The soil is deprived of its normal period for recuperation and unable to replenish its lost fertility and the immediate consequence is the significant reduction in the productivity.

Factors contributing to the persistency of *Jhum* cultivation in Meghalaya

Cultural and Religious factor

The people still practice *Jhum* cultivation as part of their tradition, their ancestors had practiced it from centuries, when there was no scientific knowledge of agriculture. Some tribes like the Aos of Nagaland have Maotsu which is an important festival celebrated after the *Jhum* fields are sown. There are some who connect this festival to the fertility of the earth and expect a rich plant growth. Wangala, a harvest festival of the Garos is celebrated with pomp and gaiety to appease the Gods. It is observed that *Jhum* is not only an economic activity but also inseparable from certain elements of spiritual ethos of the community as well.

Simple and cheapest method

The method involves minimal land preparation, insignificant capital investment, no manures or fertilizer requirement and manual labour involving only family members.

Absence of land ownership

In Meghalaya the land belongs to the community and in the absence of a secure tenure, the *jhumias* are unwilling to invest in land development activities. This lack of ownership makes the *jhumia* unable to have access to bank facilities. However, with increasing communication of life and with certain community land being released for sale, there is also every likelihood of the danger of usurpation of land by the affluent of society and consequential marginalisation of poor farmers of *jhumias* in rural areas.

Mixed cropping

The *jhumias* are self sufficient in a way because they consume the crops from their own *jhum* fields, like rice, millet, maize, fruits and vegetables so that their requirement for their daily consumption is fully met. Also, the presence of different crops in the same plot is conducive to pest management due to genetic diversity and the sequential harvesting of crops is an effective way of managing many species over both space and time and contributes to agro-ecosystem stability, besides showing better orientation of nutrient use efficiency.

Lack of improved technology awareness

In a State where ethnicity is prominent and there is general aversion to bring labour from outside, the continuance of shifting cultivation practice would largely depend on the kind of developmental activity that is being offered. The lack of creating awareness and non-introduction of improved technology that are location specific and need based would only strengthen the farmers resolve to resist change and persist with *jhum*.

Lack of Financial Support

Besides the bottlenecks of development highlighted above, one of the biggest constraints in livelihood matters relating to *jhum* control, poverty alienation, conservation oriented and other income generating programmes is the lack of extension of credit facilities. Traditional land tenure is blessed as constraining security for the credit. However, this appears to be only an excuse for non-performance and reluctance for credit extension. Therefore, with this in mind, the *jhumias* have no alternative but to continue with his age-old practice of *jhum*.

Lack of co-ordinated multi-disciplinary approach

The *jhum* control programmes are being implemented by different developmental agencies in relative isolation without proper co-ordination through multi-disciplinary approach. This lack of institutional consultations leads to mainly personality-based consultation with each trying to out do or overdo others resulting in alienation of traditional systems and knowledge from the decision making and governing process, leading to marginalisation and non-participation by the *jhumias* in the developmental works.

***Jhum* Control Programmes (Government Schemes)**

Recognising the major problems on *jhum* cultivation in the State, the Government through the Soil and Water Conservation Department took up the scheme of *jhum* control in 1974-75 under the State Plan. This scheme was undertaken to provide effective supporting base for permanent settlement of the *jhumia* families in order to wean them away from *jhumming*. The scheme is a package programme and consists of the following components:

1. To provide permanent cultivation land (Bench Terraces) with assured irrigation facilities.
2. Follow up programmes by way of supply of inputs like seeds and plants, manure and fertilisers including cultivation cost for three years at a gradual sliding scale.
3. To provide cash/horticultural crop plantations and afforestation.
4. To provide drinking water supply for village settlement.
5. To provide link/approach roads to work areas.

Land development in the form of bench terraces with assured irrigation facilities have proved most effective means in attracting the *jhumias* to settled agriculture. Cash/ Horticulture crop plantations undertaken under this scheme is also making an appreciable impact in the process of weaning away the *jhumias* from their age-old practice of *jhumming* and it could be seen in the field that most of the abandoned *jhum* areas have been converted into permanent cash/horticulture plantations such as areca nut, cashew, citrus, etc.

However, the poor extension services, lack of dedicated workers to save rural

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areas and the failure to ensure effective marketing linkages along with communication and transport network meant that the *jhumias* continue to miss the opportunities and remain isolated and backward. Besides the above, there are other factors that contribute to the overall stagnation and slow progress in eradication of jhum in Meghalaya. These are:

1. Lack of ownership right over the land.
2. Lack of inputs, improved technology and result oriented approach in interior areas.
3. Lack of integrated, co-ordinated and sustainable approach based in research and development, which will give a good impact to change the mindset of the *jhumias*.
4. Lack of proper co-ordinated efforts and effective linkages between research agencies and developmental agencies.
5. Lack of need based technology and flexibility of the scheme, particularly schemes from the Government of India.
6. Among poorer sections of the society, the long gestation period of plantation crops became a hindrance.
7. Lack of pre-implementation capacity building and farmers participation in the programme.
8. There is no continuity of Research in the *jhum* fields.

Agro-forestry as an alternative to Jhum

Agro-forestry is a composite, diversified and sustainable production system. It is a practice that has a long tradition in the North East where trees are integrated in the crop and livestock production system according to agroclimatic and other prevailing conditions. In Meghalaya, a study conducted by the ICAR in 1987 revealed that the Khasi Mandarin (*Citrus reticulata*) was introduced in agri-horticulture system with a plant diversity of 800 plants/ha and 400 plants/ha (Bhatt et al., 2000). The fruit yield was noticed after 6 years of plantation with no change or variation in plant height or canopy variation, and its production level was also quite high which shows that the Khasi mandarin could be a potential source of agro-forestry in Meghalaya. The Khasi Hills are rich in wild edible herbs and plants which form an important constituent of their daily diet where about 143 species of herbs and plants beginning with the prefix 'ja' have been documented. These NTFP products have served as a primary source of revenue to the villages especially women who sell them in the local markets. (Khongsit.1999)

Such an idea of developing alternative models in land to suit local farmer's needs for agriculture has long been advocated by scientists and scholars. According to them any alternative model can only be adopted by local people if it suits to the local environmental condition and meet the needs and constraint of farmers. Besides this, the model should be

similar to traditional practices with minor alterations. They recommended introduction of three components, i.e. agronomy, forestry and animal husbandry which will help minimizing the soil erosion, conservation of moisture, increase in infiltration rate, decrease in evaporation from soil and balance nutrient status and finally help in land remaining productive on a sustained basis. Their main contention is that “we generally try to transfer technology in the farmers field without considering biophysical and psychosocial economic problems of the farmers. The farmers never accept imposed technology under any circumstance”. (Jha and Tiwari, 2013) They are of the opinion that instead of spending time in on-station trial the extension workers should work along with farmers to examine requirements of the farmers and biophysical causes. They should conduct on-farm trial in the farmers field itself with collaboration of farmers on model basis in 5 or 10 villages in first phase. The whole process of agro-forestry being multi-disciplinary in nature it requires a team work of agriculture, forest, soil-conservation, social science and animal husbandry departments. Thus, the Agro-forestry as an alternative to jhum cultivation, is worth giving a fair trial by the departments concerned.

Conclusion

Jhum cultivation does not only disturb the ecological balance and destroy the environment but also causes a chain of affects and after affects leading to multifarious adverse conditions of soil and climate. However the efforts so far made by the various departments of the Government of Meghalaya to wean over the jhumias from jhum cultivation seem to be encouraging enough. The NERCORMP project supported by the International Fund for Agricultural Development has done commendable work in West Garo Hills of Meghalaya by assisting the jhumias in rationalization and optimization of jhum practices through community-based participatory planning, implementation, monitoring and social auditing of their jhum procedures. The project also provided additional inputs in the form of livestock, mainly pigs, and home-garden developments. A recovering fund was also established for Women’s Self Help Groups and village level National Resource management Groups (NERCORMP,2008). It is also hoped that the Government joins hands with primary departments like Horticulture and Soil and Water Conservation Departments to work towards economic upliftment of the jhumias through market focussed and subsidy based programmes. In its bid to follow the current emphasis on ‘weaning away’ hill farmers from this so called ‘primitive’ style of conservation, government policies should in turn shift to ‘supportive’ policies.

Notes

1. ‘Ja’ is a Khasi term which means ‘rice’-a staple food of many tribes including the Khasi. It has been documented that there are about 143 species of edible herbs and plants in and around Khasi-Jaintia Hills that begin with the prefix ‘ja’ which goes to prove that there was a time in History when the people sustained themselves by

consuming these herbs whenever there was a scarcity of food or a famine.

2. NTFP – Non Timber Forest Products are an important source of daily diet of local villagers and form an important source of livelihood to the rural folk who sell these products in the local markets. Some common NTFP products of Meghalaya are bamboo-shoot, mushroom, broomstick, honey, yam, edible herbs and plants etc.

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Migration of tribals and their settlement: A study in Dindori district of Madhya Pradesh

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Abstract

The establishment of multinational companies and landing development programmes in tribal areas has been pulling as well as pushing factors inducing tribal families to migrate in different parts of central India. The study was conducted in tribal dominant areas of the State. The present paper describes the impact of migration on children of the migrant parents and also to understand the migration pattern of tribal population in Dindori district of Madhya Pradesh. The present study is also including women migrants from the tribal areas to the neighbour cities of the State. The findings of the study has utilized in putting forward various suggestions which can help the policy makers to undertake various development and welfare interventions among these tribal groups.

Keywords: Migration, Development, Employment, Factors, Women, Strategy.

Introduction

Migration of the tribal population since long has been taking place in different states. Tribal people are migrating voluntarily from one state to another state in search of work and to earn their livelihood. They migrate as a poor and indigent person. It has been noticed that the nature and pattern of tribal migration has been changing during this contemporary period. Between 1950 to 1980, tribal people migrated from one place to another for doing agricultural labour (Mosse *et al.*, 1997) but in present scenario (from 1980 onwards) they have started migrating to the cities/metropolitan locations in search of some gainful employment/casual labour in the unorganized sectors and as household maids for their livelihood. In recent years it has been noticed that there is a large scale migration of single woman from tribal areas to cities for earning, which is a subtle change from earlier migration patterns when only the men migrated to urban centers. Nowadays tribal families are suffering from various kinds of problems i.e. poverty, unemployment, beggary etc. which lead to drive their unmarried daughters to cities in search of work. However, single woman and tribal girls fall prey to exploitation not only by employers but also by anti-social elements (Mosse, 2002).

There are numerous factors responsible for migration of tribals in general and tribal women in particular. Tribal migration is emerging as a dominant form in both rural and urban India. Many studies reported that large numbers of labourers migrated from

one state to another searching for work every year (Deshingkar *et al.*, 2008). It has been reported high levels of out-migration takes place among the tribals of hilly, densely forest and remote drought prone regions of Madhya Pradesh. In southern parts of Madhya Pradesh, 65% of tribal households are included as migrants' labourer (Mosse *et al.*, 1997). They have migrated to the neighbouring states to work in industry, seed cotton farms and textile markets (Kate Bird and Deshingkar, 2009).

Most of the studies reveal that the occurrence of tribal migration has undoubtedly been emerging day by day in the tribal areas of Madhya Pradesh. In development ranking, Madhya Pradesh is recognized as the least developed state in India. The state is considered as largest tribal population inhabited state. Tribals of southern parts of Madhya Pradesh consider migration as a long livelihood strategy. The recruitment of migrant workers is basically organized by the local agents, known as Mukkaddams. They provide advance cash to the labourers to help their family left behind in the absence of the migrant. The migrant labour also uses the advance to purchase some needs during their journey. The advance cash are reimbursed through wages of migrants in installment including commission of local agents. The duration of installment depends on the amount of advance received at the destination and the wages they are paid. The agent obligate the recruit migrants to stay together in a common room at the working locations. They are compelled to live in a surrounding with shelter without easy access to drinking water and sanitation. Female migrants and children are highly vulnerable to disease, injuries and sexual abuse in such circumstances. They are exposed to harassment by contractors, police and urban authorities.

It has been found that the migrants are engaged in factories as a labour, working as domestic servants, bus and truck cleaner, rickshaw pullers, street hawkers, petty traders, construction workers and house maid workers. Earning is one of the major drivers of tribal migrants in search of paid employment. It has been reported in earlier study that the internal migration can lead to positive transformation in both sending and receiving areas (Deshingkar *et al.*, 2006). Migration can help the migrants to reduce their unemployment, beggary and to halt the slide into poverty. Besides all these, a few negative impact of migration is there such as social and cultural identity, security, acute dearth of labour and high dependency ratios in sending areas. On one hand mass migration can lead to worsening poverty of migrants but on other hand, due to faraway from native place they lack their traditional rights on land, forest and voice in community decision making process. Migration can also have a negative effect on collective action and natural resource management.

Studies reveal that tribal migration is resultant of displacement due to establishment of various development projects in tribal areas. There are different forms of migration seen among the tribals such as inter-state cross migration, regional and seasonal variation, occupational migration, short term and long-term migration. Besides, these studies still have dearth of information and detailed analysis. Hence a study to understand is of importance in the migration in tribal India despite having large number

development programmes like Mahatma Gandhi National Rural Employment Guarantee Act (MNREGA), Jawahar Rojgar Yojana (JRY), Employment Assurance Scheme (EAS), Food for Work Programme (FFW), Prime Minister Gramin Swarojgar Yojana (PMGSY), Swarna Jayanti Gramin Swarojgar Yojana (SGSY). The purpose of the present research work was to find out the cause behind the migration and to document how and where they are migrating, their living conditions prior to migration, future planning of migrant tribals, awareness about the development programmes being implemented for their benefit, health and hygienic status, occupational mobility, impact of the migration on their socio-economic status, agencies involved and other aspects particularly related to the tribal women migrants. The study has focused on the tribal majority states of Madhya Pradesh. The study is also including women migrants from the tribal areas to the neighbour cities of the State. The findings of the study are expected to be utilized in putting forward various suggestions which can help the policy makers to undertake various developmental and welfare interventions among the tribal groups.

Objectives of the study

The objectives of survey is focused and aimed

- To provide a profile of the study areas and economic background of the respondents.
- To delve the strategies adopted for the development and use of assets by the migrant tribals.
- To explore the health problem and strategies adopted by the migrant tribals to cure themselves from the diseases.
- To suggest measures for the betterment and policy implication for migrant tribals.

The rationale behind the choice of the district is the following: 1) The tribals of Dindori district mostly inhabit the hilly regions, mainly in close proximity to forests comprising more than 58 percent of population. 2) The district in which almost all of the Tribal Groups live for centuries, being far away from the mainstream in their relatively isolated, inaccessible, less fertile and less agriculturally productive regions of forests, hills and mountains; 3) The traditional occupation of the tribes is mostly based on the traditional agricultural system. The government plans and policies regarding employment is not sufficient for them to meet their livelihood and collection of wood is a major source of income. 4) The district is not well communicated with the district head quarter. 5) Census provides few characteristics of migration including proportion of rural and urban migration. But it does not provide data on tribal migration. So, it becomes important to study dynamics of tribal migration. The available literature is also scanty. So, against this background a study was done to understand the migration pattern of tribal population in Dindori district of Madhya Pradesh.

Methodology

Field survey were undertaken in 50 remote tribal villages and forests areas among Baiga, Gond, Kol, Pradhan, Dhulia, Bhoomia and Agaria tribes in Amarpur and Bajag block of Dindori district of Madhya Pradesh. The district of Dindori has a special distinction of accommodating about one-sixth of the total tribal population of the State. Tribals only constitute 64% of the district and thus, it stands fourth among all fifty districts of the State with highest proportion of tribal concentration. The selection of the district was made in view of the concentration of tribal population on the one hand, and their general socio-economic backwardness on the other. No background data was available for migration among tribal population in Dindori. Tribal People of Dindori district used to migrate for earning their livelihood. The district is situated at the eastern part of Madhya Pradesh touching Chhattisgarh state. It touches Shahdol in east, Mandla in west, Umaria in north, and Bilaspur district of Chhattisgarh State in south. It is 144 Km from Jabalpur on S.H 21, 104 Km from Mandla and 88 Km from holy place Amarkantak. It is located at 81.34 degree longitude and 21.16 degree latitude. The holy river Narmada passes through the district. It is situated at a height of 1100 m above sea level amongst herbal-rich, Maikal mountain ranges. Dindori has many historical as well as spiritual places. Some of the spiritual places are Laxman Madva, Kukarramath, Kalchuri Kali Mandir etc. The Kanha Tiger National Park is 180 km & Bandhavgarh National Park is 140 Km away from the district headquarter. It was created on 25th May, 1998 with a total of 927 villages. The district is covered in seven blocks namely Dindori, Shahpura, Mehandwani, Amarpur, Bajag, Karanjiya & Samnapur. The Baiga are particularly Vulnerable Tribal Groups which can be found only in this district. The Baigas are known as the “National Human” (Official Website of Dindori District, 1998.)

The datab of the study comprised of primary data collected through interview schedules, i.e. the tribal beneficiaries and the development officials. Information from tribal beneficiaries of the selected villages was collected through focused group discussions, observation and interview schedules, information from development officials representing the specialized agencies as well as community development blocks, banks and other financial institutions through personal interviews using the interview schedules. The interview schedule was administered to collect information at the district, Panchayat and village levels. Sampling plans address certainty and precision of results by defining who is included in the survey, how many people are needed and how respondents are selected. The researcher has conducted his study in Amarpur and Bajag block of the Dindori district. Under these blocks 20 GPs were selected (10 GPs were in each block). It was decided that 250 sample households would be interviewed covering all the 50 villages from the two blocks of the districts and equal number of samples taken from each villages with an average of 5 respondents to be selected at random (including both migrants and non-migrants households). 135 men and 115 women were interviewed. Data were collected from selected respondents through multi-stage random sampling procedure. The selected sample respondents were contacted in their respective residence

and required primary information was collected. Primary data were collected through interview schedule of structured and quite a few unstructured questions and focus group discussions related to tribal migration and interface. During the collection of the data some important aspects like economic conditions, livelihood pattern, use of assets, health and hygienic conditions, educational status of the children of migrants, social policy and State services for migrant's development were also focused. Information in respect of migration and change, land acquisition of the beneficiaries were also obtained.

Secondary data were collected from various sources and a major part of the analysis of the study is based on the secondary data collected. For secondary data, official perspectives and feedback on tribal migration, the researcher primarily engaged with the government department, Tribal Welfare Department, Report on selected statistic, Published by National Census and the National Sample Survey and Ministry of Statistics & Programme Implementation, Government of India, New Delhi were consulted. Secondary data collection sources also included government offices at the State and district level, National Rural Livelihood Mission Offices (NRLM), Tribal Welfare Department at the State and district level through the ITDAs. Several field trips were undertaken for collection of data during different seasons. Information was gathered through oral interviews of the local tribal people. These data were analysed quantitatively or qualitatively depending on the objectives and design of the study.

Economic profile of the migrants

The indigenous people (Scheduled Tribes) have their own distinctive culture, residing in a specific geographically isolated area with low socio-economic conditions. For centuries, the tribal groups have remained outside the realm of the general development process due to their habitation in forests and hilly tracts, and within the close proximity of nature. After independence, Government of India has scheduled the tribal groups in the Constitution and provided special provisions for their welfare and development. There are about 654 ST communities across the States in India and 75 of the STs are backward and are termed as Primitive Vulnerable Tribal Groups. Most of the tribal areas of the country are hilly, inaccessible undulating plateau lands in the forest areas resulting in the bypassing of general development programmes. Due to this, the infrastructure and development facilities in tribal areas for education, roads, healthcare, communication, drinking water, sanitation etc. has lagged behind when compared to other areas which has resulted in further widening of the gaps of development between the tribals and the general population.

Table-1: Monthly earning pattern of the tribals

Average monthly earning (Rs)	Frequency (Number of tribals)	Percentage (%)
Upto Rs. 2000	17	7
Rs. 2000 – 4000	35	14
Rs. 4000 – 6000	38	15
Rs. 6000 – 8000	12	5
Rs. 8000- 10000	128	51
Rs. 10000 -12000	15	6
Above Rs. 12000	5	2
Total	250	100

The study revealed that 51% of tribal migrants are earning Rs. 8000- 10000 whereas least numbers (2%) of migrants are earning above Rs. 12000 (Table 1). Most of the tribal migrants reported that their existing monthly income is better than the old incomes. It is seen from the income pattern that one third of their income is from migration related works.

The economic background of the tribals is primarily based on hunting, domesticating the animals, poultry farm, buffalo, goattry, collection of fire woods and forest products, fishing and practicing the shifting cultivation. A very small number of tribals are engaged in non-agricultural activities such as mendicants, pastoralists leading a semi-nomadic to nomadic life. The forest laws have curtailed the free movement of tribals in forest regions. The tribal rights on the forest lands have been severely affected. Shifting cultivation is regulated by restrictions on the use of forests. Use of Minor Forest Produces (MFPs) by tribals is reduced to a large extent. Exploitation by money lenders and contractors, problems of credit and market for Minor Forest Produces (MFPs), poverty, hunger, malnutrition and impoverishment are important evils, which the tribals have been facing since long time (Misra, 2000). Land alienation and displacement are other major problems prevalent in the tribal areas.

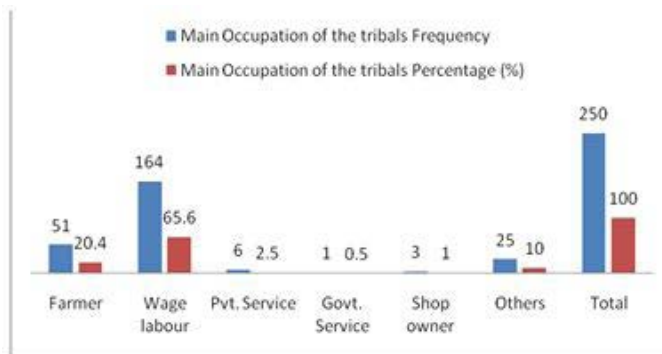


Figure 1: Occupation categories of the tribals

Tribals are engaged in various occupations but the major occupation is rain fed agriculture. The main yields in the farms are millet, rice, rai, til, macca etc. Only Kharif crops (crops grown during summer season) are grown due to unavailability of water throughout the year except in rainy season which is brought by the monsoon. The main crops grown is millet. Few of them work as labourers and few were engaged in subsidiary occupation like carpenter, making of sal plate, making of basket etc. For that they collect material (sal leaves, bamboo, wood etc.) from the forest. During these collections, they are trapped by the staffs of forest department leading to curtailment of their supplementary livelihood income.

Majority of tribal's family belong to Economically Weaker Section (EWS) income group. Monthly incomes of the tribals are very low, because large numbers of the tribals are landless labour and do not have agricultural land. The annual income is very low. Those who have some agricultural land with domesticated animals have elevated their economic standard. Respondents are of the opinion that agriculture, small cottage industry and industrial ancillary jobs are the main source of their income. Thus from the data, it is evident that large number of tribals' main source of income is agriculture.

Tribal society is largely an egalitarian and tribal women put their hand equally with tribal men in their contribution to domestic economy. Comparatively they do more physical work in their agricultural fields and forest. Usually they have enjoyed a privileged social status in their own communities than women of other communities in general. The economic status of tribal women is relatively low in comparison to tribal men and general population which is also associated with their poor nutritional and health status. Despite having their routine household work, they do hard labour in the agricultural field and forests for long hours. Even their schedule of working hours continue during pregnancy, natal and postnatal stages. Thus, they face high morbidity rate, and low child survival rate. The tribal women also suffer from different kinds of taboos and superstitions and are away from the benefits of existing development programmes and welfare activities.

The livelihood pattern of the migrant families

A large quantity of natural resources with rich minerals is depository in tribal

areas. The ongoing development projects i.e. hydro-electric power generation, industrial expansion, opening of mining activities etc. exploit the natural resources which lead to deforestation, destruction of environment, displacement of the tribals and also alienate them from their land and grab the basic right of livelihood from the forest. Massive investment in construction of dams, power plants, industrialization and mining create wealth to the nation and employment opportunities to various people but all of these have hardly given any assistance to the tribals and rather creates difficulties for tribals. As per the Ministry of Rural Development, large numbers of tribals inhabiting in forest areas for generations together have been deprived of their lands and traditional rights. Due to the enactment of Forest Rights Act, 2006, there is a provision of allotment of forest land to the Scheduled Tribes and other Traditional Forest Dwellers by following certain procedure which enables the state Government to do justice for forest Dwellers by providing forest lands. Even now large numbers of tribals in the study areas are struggling to get the land patta. Many a times they have raised their issue related to the forest land in the gram sabha but the assurance of the sarpanch and sachiv were not satisfactory. Lack of relief from this have motivated them to migrate to neighbour districts, towns, cities and state for work.

The conducted study comprised of areas where the villages are surrounded by forest of Sal trees,. The water of Sono River brings fertility to land, where the farmers can generate lot of production on their land. They have adequate agricultural land but could not produce the agricultural output. Tribal farmers concentrated on their agricultural land and hoped to produce lot of grains. They have to do hard labour to fulfill their basic requirement. The area is rich in biological diversity, which may promote eco-tourism. The coming of tourist can develop the area. The tiger, forest and folk culture of tribals may attract the peoples. More than 90% of the tribals, to a large extent depend on forests and forests resources for their livelihood. They collect small forest products such as *Harra* and *Baheda* (local name) in the month of March, April and May, *Amala* (local name) in the month of October, November and December, *Tendu Patta* (local name) in the month of March, April, May, June, November and December, *Dhup* (local name) in the month of January, February and December, *Honny* (local name *Saheda*) in the month of January, February, March and April, *Chironji* (local name) in the month of May and June, *Mahul Patta* (local name) in the month of March, April, August, September and October. All the above forest products are collected by them and sell in the weekly village market at very low price. They sell it to the small businessmen. These are the secondary source of income of the tribals. Another secondary source of income of the tribals is domestication of cow, buffalos, goat, pig and poultry etc.

The living conditions of tribals do not reveal one's right to life and a dignified living enshrined in the Constitution. Families make their homes under trees in or near the fields. They have dilapidated roof or walls. The clothes are hung on the wall of their roofs. The ropes are tied to make a swing for kids. In the rainy season, the rain water enters their house. Children able to walk wander around in hot sun mobilize water and fuel woods

and are devoid of proper care and food. Most of the families subsist on mere roti and chutney and occasional supplement of dal. Inadequate food, nutrition, healthcare lead to rapid malnourishment. Tribals are unable to spend small amount in private expenditure involved in procuring teaching material and clothes due to weaker economic condition. Under such circumstances expenditure on non-food items is not of primary importance in the struggle of tribals for their survival. The importance of food collection is felt more important for tribals and to be engaged in household works like tending for the younger siblings in the absence of parents, grazing cows, buffaloes, and also supplement family income as child labour to earn small wages which strengthen their family status. The families live in darkness of night. The fear of wild animals scares them. As the sheaves and husk are highly inflammable, even in some study areas electricity facility is not available and they use oil lamp light. There seemed to be no efforts by the local administration to provide any basic facilities to these tribals. They have no accessibility to school, anganwadi, health center or fair price shops. The tribals families in fact are forced to move from one place to another place for job.

Migration for survival

Table 2: Number of migrants over the decades in India

Decades	Migrants (In millions & Percentage)
1951-61	144.8 (33%)
1961-71	166.8 (30.4%)
1971-81	203.5 (30.6%)
1981-91	230 (27.4)
1991-2001	307 million (30%)
2001-11	453.6 millions (37.8%)

Sources: Census of India, 2001 & 2011 D-Series Migration table

Migration is an age old practice but it is increasing at a faster pace over last decades due to variety of reasons. In recent years it has been observed that there is a high migration rate in India. According to the National Census for 2011, 453.6 million (37.8%) of the population were migrants (Table 2). The above data reveals nearly above a one-third had migrated during the previous decade.

The tribals in the study areas engage themselves on their agricultural farm only for five to six months and they do not have any work during the remaining period of the year. So, they migrate to neighboring districts of the State. These districts have large yield of work in the industry or mines and there is a shortage of labour in those areas. So, they call both men and women for work in the mines. Agriculture is the main occupation of people in this area. They do farming but the yield is less. After doing hard work when the yield from the crops is less and insufficient for their survival, it becomes difficult to live in the villages. That is why they have migrated to other areas in search of jobs. They return

back to their villages after two or three months. The entire tribal family members do not migrate, few of them stay at home to look after the aged and children of the family. In the study areas it was found that only male members who are capable to do the work have migrated to their neighbouring cities, districts and State. Large number of tribal people from villages migrate to different areas of neighbour districts during the lean period of the year. Generally the millet and rice yielded in the farm is kept for eating. Some tribal people have to go to the forest for collection of honey and forest products. They roam in the jungles and stay there. Traditional tribes are also involved in hunting, fishing and collection of chironji. Though it has declined over the period, but still some PVTGs tribal people are involved in this business and they go to market for selling. Earlier wood was available in the forest on large extent. Now they don't go to collect wood as the forest cover has declined. Hence, they only do farming. Earlier they used to make wheels of bullock cart, tatty etc. out of the collected wood.

Migration for education

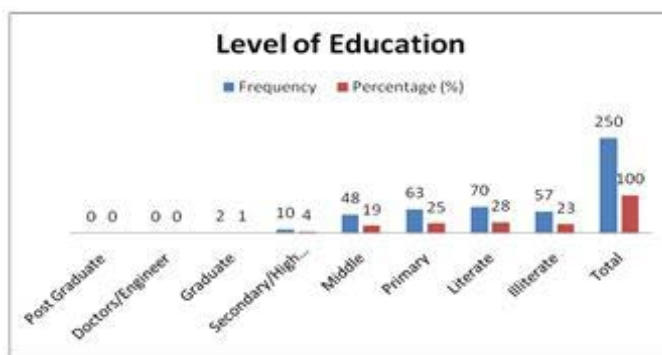


Figure 2: Educational levels attained by tribal migrants

In the surveyed villages it was found that not a single case of professional degree or post graduate level studied. Tribal families have reported that they have studied up to middle and high school level which reveals that educational services are not equipped in the interior tribal areas (Figure 2). In most of the study villages education is available till 5th standard (primary level). For further studies tribal children have to migrate to nearby town and cities. Tribal parents have an ambition to send their children to the school so that they would get employment, knowledge of business, moral education, empower etc. Earlier, tribal girls' proportion in education was less as compared to boys but now they have also started getting educated. It was observed in the field work that a number of tribal parents understand the advantages of education. Parents are also interested to send their children to schools and colleges for study. After 10th class they have to go to cities. The proportion of children going to cities is very less. Children return to their villages during festivals and vacations. Parents are aware of the importance of educating their children. They wish that their children should study well and stay happily. They felt that it's their responsibility to send children to schools. They earn Rupees 150 to 200 per day. If the children are interested in their studies then they send them to school. Otherwise the

parents ask them to go to the agricultural field. Overall awareness about the importance of children's education is increasing and the tribal people are coming forward but yet their proportion is less. Still the primitive tribes are comparatively less aware about the importance of education. One key informant explains reason for it as: it's like growing trees. If one gets educated others take inspiration from them and go. But when there is no tree at all, how can other trees grow?. It implies that nobody from primitive tribe took initiative in getting education. That is why subsequent generation is not inspired for education. Though awareness in education has increased but it is still in its infancy. This is the first generation which is studying in secondary schools and colleges. Proportion of children going to the college is still less. Most of the children stay in the hostels provided by government and few of them stay in rented rooms. Children till 10th class get hostel facility in the school itself. But children going to the college in cities stay by hiring rooms. Very less proportion of children go to private schools for studies since they can't afford costs of private schools. Some Non-governmental organizations are also trying to raise the educational status of tribal by providing education to drop out children. So the study reveals that most of the tribal children are moving towards cities and town for getting higher education and with a high ambition of employment.

Migration as a strategy

For many chronically poor tribal people with few assets, education or social connection, migration has become an important way of coping with seasonal fluctuations in income. Where agents or middle men (sometimes women) are involved, earnings can be limited and working and living conditions can be basic. Olsen and Ramanamurthy (2000) has documented how the migrant labourers are exploited by contractors or recruiting agents in a varied of subtle ways and they range from trapping them into a bonded labour by paying lower wage, extracting to overtime work and child labour and using caste-based and patriarchal modes of oppression to maintain abusive labour relations. The system survives because recruiting agents are seen as those who save the labourer in distress by offering work when otherwise they might starve. Some lower-caste people who serve the landlords and employers think, they will be rewarded with patronage during crises. This patronage may consist only of loans, which further bind the worker and the worker's family.

This kind of migration rarely results in the accumulation of assets. Such migrants often migrate through an agent who takes a heavy cut of their earnings, and this is one of the reasons for them not being able to break out of poverty. However, the importance of such migration should not be underestimated, because in the absence of local opportunities for employment and enterprise, it provides earnings which prevent downward slides into poverty. Without this option, the poor would be dependent on local moneylenders and face starvation. In fact, this is what the poor say, migration is not easy or an ideal way of earning money but it saves them from starving to death and begging for food.

For those with better social networks, marketable skills and more education and/

or assets, migration has become an accumulative strategy. The tribals were traditionally skilled stonecutters and well diggers. They have adapted this skill to dig trenches for telephone cables, carry out road works, and have now become well known all over central India. In tribal areas, they have benefited from public works executed by gram panchayats and state agencies through schemes for tribal water supply, housing, food for work, watershed development and the construction of schools, public buildings and offices. They work almost all year round but the nature of the job varies by agricultural season: forest department work is undertaken in the dry season and road works and trench digging are done in the rainy season. Both the poor, non-poor and all landless households have migrated. Groups of tribals have migrated together and go for 15-30 days at a time. They make two to three trips in a year. Each group is headed by a contractor, usually a tribal, who bears all travelling and food expenses. The migrant tribals may have taken an advance payment from the contractor to transfer of funds to their family. Later, he cuts this from the wages of the tribals. Earlier, contractor would be the main source of information about new jobs and wages but over time their power has eroded and they now play a more facilitating role rather than controlling and exploiting the tribals. These days, most of the tribals do not have fixed contractors and work for the person who makes the best offer. It is evident that the tribals have done well for themselves out of migration, individually and collectively. Many contractors have pucca houses with all kinds of durables things (such as- TV, fridge, LPG gas stove, and motorcycle). On the other hand, the poor migrant tribals have dilapidated houses. They have invested their newly acquired wealth in building their house, well, schooling education of their children and any other household activities. Perhaps it is because of their economic power that they have also gained a strong presence in the gram sabha, despite being absent frequently and belonging to a lower caste.

Health problems of native and migrant tribals

According to the local inhabitants most of the diseases are water borne starting during the month of August and September. During the last few years the government has started paying attention towards these tribal areas. Many new health schemes like emergency service, mobile clinic and new insurance scheme etc. have been initiated by the government in recent times. Some of the respondents view that they migrate to neighbouring districts and state for work. If they fall ill at the destination place, then few of them consult a doctor or take traditional medicine to cure from the diseases. But few of them also refer to return to their native place. People go for work to their farms which are far away from their houses. So, they carry food and limited water with them. But the drinking water is not sufficient for the whole day. So, they drink contaminated water available in the local ponds, tanks, springs etc. and fall sick. In most of the villages there is no provision of safe drinking water.

Earlier tribal people did not use fertilizers and chemicals in agriculture. Nowadays they use chemical fertilizers like DAP and urea in their agricultural farms to increase their production. Now a days they take hybrid grains for growing their production. These are

yielded with the use of chemicals and fertilizers. With that the incidence of diseases is also increasing due to chemical mixed food. In the past if they get fever then it used to get cured immediately. It is believed that they used to pray God after which they get cured. But now, the respondents say that this does not happen now. During that time the incidence of disease was very less. Earlier if somebody got disease then he used to take herbal medicine and got cured immediately. Now-a-days if one falls sick, everybody gets infected by it. Now-a-days new diseases are occurring. Oil was also extracted from the seed using manual machines. Now they get it in packets which are processed in industries. If any disease persists for four days or more then they go to the doctor and take treatment. Government doctor does not remain available in most of the times due to lack of facility in tribal areas. In the study areas it has been observed that it is a dream for tribal to get a better treatments like injections, saline, tablets for cure. The major health problems faced by the tribals in the study areas are fever, vomiting, loose motions and stomach pain. Tribal people don't take immediate treatment for any ailment. Still many people prefer herbal medicines since it remains available in the vicinity. If not then they go to the nearby public health facility. During last years the incidence of the diseases was more. People suffered from vomiting, loose motions and malaria. Generally, people seek health care after three to four days of the onset of the disease. Primary Health Center (PHC) is available at the district head quarter and Primary Health Centers available in the tribal areas do not have doctors availability. The people are then treated by nurse and compounder. In case of serious conditions, the patients are either taken to the Community Health Center (CHC) or to the District Headquarter hospital which is 60-70 Km. Many tribal people also seek health care treatment from the ARMPs (Ayurvedic Registered Medical Practitioners) or quacks. Many ARMPs visit the villages and give medicines and charge Rupees 50 -70 from each patient. The government health facilities remains far away and most of the times there is absence of doctor or other staff. So, the people prefer to go to ARMPs, who are readily available and also charge less money. But the ARMPs are not properly trained and also do not have valid qualification for treating patients.

First aid facilities are not available in the work site for the tribals. Tribals work in extremely iron and bauxite areas. As there are iron and bauxite, the possibility of tetanus is high. Most of the tribals in the study areas are working in the mines. In case of major accidents like loss of limbs the employer gets the worker treated and sends them back with some paltry compensation. There does not seem to be any role of the State administration in this regard.

Table 3: Composition of tribal population under study

Name of the Tribals	Frequency in Household Wise		Percentage (%)		Total	Over all Percentage (%)
	Men	Women	Men	Women		
Gond	23	16	9.2	6.4	39	15.6
Agaria	19	15	7.6	6	34	13.6
Dhulia	18	17	7.2	6.8	35	14
Pradhan	20	16	8	6.4	36	14.4
Bhoomia	18	16	7.2	6.4	34	13.6
Koal	19	15	7.6	6	34	13.6
Baiga	18	20	7.2	8	38	15.2
Total	135	115	54%	46%	250	100%

Table 3 shows the composition of tribal communities . It has been observed that they face various health problems in these areas. The tribals migrants also reported that there are many service providers working in these areas to solve the health problems but it does meet their demands. The need is very high and Government service providers could come handy in providing the support. Thus tribal people need special attention for improving their general, reproductive and child health status.

Migration and change

Migration give tribal people an exposure to outside world. They learn the language, customs, and traditions of other surrounding cultures. They develop desire to educate their children. Earlier they used traditional agricultural instruments on their agricultural farms which were made up of wood but now they use modern techniques that are made up of iron. The impact of mass media and communication, modernisation and globalization also gave them exposure to the outside world. They are learning new techniques as well as acquiring the cultures of others. Tribal people work in their agricultural farm for about half of the year. But when agricultural season is over and there is no work, they migrate to neighbouring areas for getting employment. This helps them to continue their income and improve their economic condition to some extent. Earlier tribal people were largely dependent on forest for earning their livelihood. They used to eat fruits, roots, mahua flowers and any other forest products. But in the present days due to the diminishing forest resources and restriction by the forest department/government in cutting trees, collection of fire wood and fuel, collections of forest products etc., the livelihood pattern of tribal community has been restricted. That is why they move in search of alternative means of livelihood.

Generally elder tribal people remain busy with routine work. In the morning they go to agricultural farm after taking breakfast. Male members of the tribal family go to field early in the morning. Women after completing their household works go to the farm by carrying lunch with them. Tribal people work whole day in the agricultural farm and return in the evening. In the evening, they listen to radio. Elder people are not interested in listening to Radio. Rather they sit together and engage in gossip. Now a days Television has become common for tribal people, where the electric facility is available. So, those tribal people having Television get opportunity to watch movies. Most of the people watch Hindi movies. Elder people watch religious movies while young people watch commercial movies. Children go to school in day time and after coming home in the evening they play games. Usually elder persons don't go to distant places but youths go to other places to watch movies. They have to go to nearest film hall for watching Hindi movies during the festival and holy occasion. Now-a-days VCDs are easily available due to which people are also watching movies at home.

Due to the influence of mass media and communication, tribal people have become more aware of the events happening outside the village. The younger generation is more influenced by the movies. Young boys and girls have even got married without the consent of the parents and resistance from the families. These marriages were inter-tribal marriages and village people accepted them later on. Elders stated that young people watch television till late night. So, their life style, food habits and sleeping timings have changed. By watching the movies tribal people are influenced by Hindi language. Now impact of Hindi language is seen over the original tribal dialect. The dress pattern of tribals has also been changed due to the influence of mass media and communication. Few young boys roam in the village by changing their hairstyles. Comparatively less influence of movies is seen among the girls.

In this study it was found that the tribal girls move to their husband's house after marriage which is very common among all tribal groups. Educated people look for legal age of marriage of boys and girls. But if parents are illiterate, then they go for their children's marriage at an early age. If the groom is economically weak then he stays at his in-law's place for some period and work in their agricultural farms. Afterwards either in-laws give him a piece of land and he settles down there permanently or he goes to his own native village. Along with character, economic situation of the family is taken into consideration while choosing brides and grooms for marriage. Both arranged and love marriages are found to be practiced among the tribal community. In the study, it was also found that lot of changes have seen in case of rites and rituals of tribal marriage. Awareness comes out among the tribals through migrations. When they have migrated to other places, they come in contact with the people of different culture, higher education, mass media communication, modernization, globalization, and technologies etc., which affect their entire social and cultural life.

Development in tribal areas

The tribal families were asked about the awareness and benefit availed by them from popular schemes of the government which revealed that some of the respondents are aware of the schemes and some even if aware are not able to take the benefit of the government schemes (Table 4).

Table 4: Awareness and benefits about the development schemes

Government Schemes	Awareness about the development scheme		Benefits about the development scheme	
	Total numbers out of 250 respondents	Percentage (%)	Total numbers out of 250 respondents	Percentage (%)
IAY	190	76	40	16
SGSY	00	00	03	1
MNREGA	238	95	215	86
RSVY	00	00	00	00
RGVY	00	00	00	00

Planned economic development was adopted as India's policy, soon after independence. Planning for the development in tribal areas was thus ushered in as part of a wider National strategy. To bridge the socio-economic gaps between tribals and non-tribals and also for the all-round development of the tribal groups, several schemes and programmes are being drawn in the Post-Independent period. Both Central and State Governments have been spending crores of rupees, in the name of tribal development, but the impact of the programmes is not as envisaged. The developmental efforts have not succeeded in bringing marked change in the conditions of most of the tribal communities. The benefits of the tribal development are not reaching the poor tribals. In many parts of the country, the non-tribal groups are getting the tribal tag, in the process, depriving tribals, by using benefits of tribal reservations and Constitutional safeguards. No efforts were made in the formulation of schemes in accordance with the needs of the tribal communities, who had their own social and cultural milieu. In recent times because of large scale industrialization and urbanization, relative isolation of the tribal areas is broken down. Due to the establishment of the State and Multi National Companies (MNCs) sponsored developmental projects (such as large scale irrigational projects, dams, reservoirs, mining, sanctuaries, industries and tourism projects and the acquisition of lands by the State etc.) in tribal areas, the tribals and their available natural resources are now exposed to the exploitative market forces.

Land acquisition and public purpose

Table 4 presents a summary on processes through which tribals have lost access to land in Madhya Pradesh

Table 4: Loss of land access by the tribals in Madhya Pradesh

Loss of access to their land/land rights and landlessness				
Loss of land thorough private transactions	Loss of land thorough displacement	Loss of land thorough through survey and settlement	Due to landlessness	Declaration of reserve forest
1. Illegal sale of land 2. Encroachment by non-tribals 3. Land mortgaging	1. Loss of patta through land acquisition 2. Lose of Govt. land cultivated by tribals	1. Cultivation land categorized as Government land	1. Poor distribution of government waste lands	1. Cultivation areas categorized as forest areas 2. Poor settlement rights during reservation

The land acquisition Act of 1894, introduced by the then British Government, still continues, with some amendments in 1967 and 1984 and this Act facilitates the Governments to acquire the peoples land, by compulsion for both the public and private purposes (Swamy, 1996). This Act helps the State as a weapon for immediate land acquisition. In most of the cases, the Government officials have been paying the compensation to workers after prolonged time, where as they acquire the lands with one notification and with in no time, but the Land Acquisition Act did not say anything on the type or nature of compensation payable to workers and time period for payment of compensation (Upadhyay, 1998). Land acquisition in Scheduled Areas are not possible, as there are number of Protective Land Laws, Legislations, Regulations, Forest Laws, Government Policies, Court Orders and Judgments, and Government Orders (GOs) which prohibit the land transfer in Scheduled Areas. In spite of all these protective and welfare laws made by the government for the welfare of tribals, the Governmental agencies have been acquiring the tribal lands in the name of National interest in contravention to all the Constitutional provisions. It indicates that all the agency laws are being manipulated where the legal access to tribal lands and resources is denied.

It is clear that displacement involves injustice to the tribal people and gross violation of their constitutional rights. The tribal people can't be deprived of their right to life merely by paying some money at the cost of their livelihood. In fact that whole legal frame itself is wrong in which the right to life of the people and their survival as community is being violated. At times the people are being forced to move out of their homes on the strength of payment of compensation. Right to life of some persons cannot be compromised or bartered just for providing more comforts to many people. Many

workers are still awaiting cash compensation promised by the project authorities. Very few workers have been fortunate enough to get permanent employment in the projects. Reports of several studies sponsored by the World Bank also admit that poor rehabilitation is not a matter of the past. In fact, in 1993 during the presence of the World Bank executed study team in the Singrauli region, a number of houses were bulldozed and workers were forced to move without adequate arrangements, and this was documented in its main report (World Bank, 1993).

Conclusions and policy implications

The empirical findings and the secondary material reviewed in this paper show that migration is higher in remote tribal areas and where the tribals are chronically poor. An important finding was that permanent migration constituted a small proportion of total movements for work and migration was the most important form of mobility. This demonstrates powerfully the inadequacy of official statistics in capturing the mobility of the poor tribal. In remote tribal villages, migration involved both the poorest and the richest households. In the case of the poorest, they are not able to sell their labour power due to old, sick or disabled; this is because they do not migrate. In the case of the richest, the reason for not migrating is that they can live comfortably from farming and/or other enterprise i.e. engages them as a labour in mining, MNREGA and other welfare schemes of the State and Central government. The broad base of migration has resulted in its benefits accruing to a large number of tribal households. Earnings of migrant labour are raising higher proportion of tribal's household income, (one person in a tribal households working outside the village). Migration is critical to manage risk and smooth consumption for a majority of chronically poor households living in remote tribal areas. The additional income of migrant labour has allowed their family to eat regularly, pay for healthcare when needed and spend on festival and social events. Migration has improved the creditworthiness of tribal families left behind in the village and it is also helpful to obtain large loans easily.

Those beneficiaries have few assets, education or social networks, migration provides a way of survival, providing income for consumption and paying off debt. Those tribal people have more skills, social connections and assets; migration brings an enough cash to set the household on an upward accumulative trajectory that is eventually led to an exit from poverty. The evidences from the study suggest that migration bring an improvement in the lives of tribal migrants. Its increase household income of migrants and it also effects on the entire village, through improved agricultural productivity and more economic activity. However, further resurveys would be needed to develop a more detailed picture of the impact of migration on sending villages. The complexity of the migration process is that its costs and benefits must be viewed against the overall social, political and institutional context of tribal areas. While it is obvious that migration is not an ideal or easy way of earning money and improving the living standard of the family, it is often the only option in places that have suffered from log jams of disadvantage. The remote tribal villages in the study areas have indeed suffered from some combination of

poor governance, leakage and corruption, social exclusion, physical isolation, restricted access to natural resources and low rainfall.

The expenses and risks of migration are very intense, including the threat of disease, injury and not being able to send their children to school. Despite of having reluctance the migrant s labour are compelled to sacrifice their children's future and their own health because they cannot take care of themselves or their children properly when they migrate. The mistake is not lies with migration but with the institutional and policy environment. The case histories also show that the distinction between employment, bondage and trafficking for chronically poor and socially excluded groups is blurred. Policy responses need to be developed that can help the most vulnerable migrants, not only migrants who are working legally. The emphasis of policy should be on minimizing the costs and risks of migration and maximizing its returns. There is a need to provide information regarding jobs, wage rates and about their rights to the migrants. There is also a need to create awareness among the police and other government departments who view migration negatively. There are now a number of NGOs and Welfare societies in the study areas providing migrant support, but they operate on a small scale with limited funding.

The future of the migration is uncertain. It will continue as long as regional inequalities persist, but the pace of development and urbanization is now increasing in sending states, so it is very likely that commuting will increase rapidly. Tribal people may eventually be able to live in the village and work in nearby cities and agricultural farms. Until such a time, it is imperative for policy to recognize the importance of mobility and migration, in particular for sustaining the livelihoods of the poorest tribal groups living in remote tribal areas. Migration has taken place vastly in the study areas. Comparatively male migration is higher than the female migration. Children have also migrated with their parents. Tribal children under the age of six are malnourished. The school going age children are avoiding of primary education, due to migration. The minimum wage of daily worker is to be Rs. 150of total amount. The work site facilities are negligible and the families live in a most inhumane condition. There is practically no privacy or health facility for tribal women. The problems for pregnant and lactating mothers are even more. There is no social security for the migrant labours. The wage earners are exploited in terms of wages payable. Hunger seems to be the most crucial factor leading to migration. Despite job cards the absence of work in resident villages has devoid these migrant families of their right to work under MNREGA. Local administration seems to be unconcerned about the welfare of migrant labours.

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Evaluation of Antioxidant properties of Quercetin in DEN-induced Hepatocellular Carcinoma in BALB/c mice

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Abstract

This study aimed to determine the antioxidant properties of Quercetin in diethylnitrosamine (DEN)-induced Hepatocellular Carcinoma (HCC). HCC is one of the most common malignant tumors in the world. DEN was used to induce HCC, and mice were divided into three groups, each having five animals. Group I served as normal control, group II received DEN (20mg/kg BW), and group III received DEN along with Quercetin (50mg/kg BW), respectively, for 6 weeks. Antioxidant potential was evaluated by employing in vitro and invivo assays. In the invitro study, Quercetins howed high radical scavenging activity with $IC_{50} = 19.17$ and $36.22 \mu\text{g/ml}$ for DPPH and H_2O_2 scavenging activity, respectively. In the ferric reducing assay, the absorbance was found to increase with an increase in the concentration of the extracts revealing reducing power. In-vivo studies revealed that Quercetin significantly ($p \leq 0.05$) retained oxidative liver markers (CAT, SOD, GSH, and AChE) activity to about normal levels. These results indicate the excellent antioxidant and hepatoprotective properties of Quercetin against oxidative-stress in DEN treated mice.

Keywords: Hepatocellular Carcinoma (HCC), DEN, Quercetin, DPPH, H_2O_2 , liver oxidative markers.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common and a major form of liver cancer and it is the ninth leading cause of cancer deaths worldwide (Center for Disease Control and Prevention (CDC), 2010). Multiple factors including chronic liver disease, cirrhosis, chronic HBV (hepatitis B virus) and HCV (hepatitis C virus) infections, autoimmune hepatitis, chronic alcohol use, obesity, and diabetes mellitus, etc. (Yang and Roberts, 2010) are responsible for its development. HCC progression may differ depending on diverse factors and therefore, several mechanisms might be involved such as loss of cell cycle control, loss of senescence control, dysregulation of apoptosis etc. (Alotaibi *et al.*, 2016; Singh, 2018). Direct or indirect exposure to radiation as well

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as exposure to chemicals such as aflatoxins, 2-acetylaminofluorene, DEN etc., leads to several genetic and epigenetic changes such as chromosomal deletions, rearrangements, aneuploidy, gene amplification, mutations, formation of DNA adducts, DNA strand-break, modulation of DNA methylation, and modulation of cell signaling pathways which ultimately leads to cancer (Villalta and Balbo, 2017; Basu, 2018). Several carcinogens are commonly used for inducing cancer in experimental animals (Biswajit *et al.*, 2012). In this study, Diethylnitrosamine (DEN) was used to induce Hepatocellular carcinoma (HCC) in mice. DEN is a Nitrosoalkyl compound that is commonly used as an initiating agent for inducing liver cancer in experimental animals. DEN, a potent hepatocarcinogen is present in cheese, soybean, fish, cured meat, alcoholic beverages, groundwater having a high level of nitrate, tobacco smoke, agricultural chemicals, cosmetics, and pharmaceutical agents (Yurchenko and Molder, 2006). It is also produced from the metabolic processing of drugs such as chlorpromazine, methadone, chloroquine, primaquine, and phenacetin (Gupta *et al.*, 2010; Ryo *et al.*, 2012). DEN induces liver cancer by forming DNA adducts where it causes methylation of the N⁷-atom in the guanines of nucleic acids and inducing chromosomal aberrations and micronuclei in the liver. Due to the carcinogenic properties of nitrosamines, the application of these substances, and in particular DEN, has become highly attractive for inducing liver tumorigenesis in rodents as an experimental model of human hepato-carcinogenesis (Swann and Magee, 1968; Tolba *et al.*, 2015). Due to its asymptomatic nature, it is very difficult to diagnose HCC at early stages, and in most cases, it is detected at an advanced stage, which is incurable. Sorafenib, a multikinase inhibitor enzyme is the first targeted therapy approved for the treatment of advanced HCC (Gauthier and Ho, 2013). Other includes, radiation therapy, chemotherapy; however, a high number of recurrences have been reported, and also several side effects are associated with all of these above modalities (Daher *et al.*, 2010; Chun *et al.*, 2015).

Owing to several side effects, plant-based products received more attention for scientific researchers as they have fewer side effects and less expensive than current treatment methods. Herbal medicine has been used as a primary source of medical treatment by traditional practitioners since time immemorial (Maqsood *et al.*, 2010). Plants are used in medical treatment because of their natural therapeutic properties, which triggers researchers to develop techniques for investigation of their medicinal properties and their potential prevention or treatment of diseases including cancer. Several phytochemicals have been studied and also used as a cancer chemopreventive and treatment agents. Some examples include apigenin, curcumin, crocetin, cyanidins, epigallocatechingallate, fisetin, genistein, gingerol etc. (Hu *et al.*, 2012). The antioxidant/free radical scavenging properties of many medicinal plants such as *Panax ginseng*, *Lagerstroemia* (Saumya and Mahaboob, 2011), *Carthamus tinctorius* (Mandate *et al.*, 2011) and *Trichodesma zeylanicum* (Frank, 2013) have been studied. Since anticancer properties of plants and their products are due to their antioxidant/free radicals scavenging properties, therefore, evaluation of their antioxidant/free radicals scavenging properties will give an insight into their anticancer properties.

Quercetin (3,3',4',5,7-pentahydroxyflavone) is a flavone from the flavonoid group of polyphenol. It is abundantly found in apples, red grapes, onions, raspberry, honey, cherries, citrus fruit, and green leafy vegetables (Hashemzaei *et al.*, 2017). Many recent studies have found that Quercetin exerts various biological effects, including antioxidative, anti-inflammatory, antitumor, antiviral, and apoptosis-inducing effect (Mandate *et al.*, 2011; He, 2016). Quercetin inhibits cancer cell proliferation by causing cell cycle arrests like G2/M or G1 arrest in different cell types and also mediates apoptosis (Seufi *et al.*, 2009). It also promotes intracellular ROS-scavenging enzymes such as SOD, CAT, glutathione peroxidase, etc., thereby reducing intracellular ROS level (Li *et al.*, 2014). Therefore, the present study was aimed to evaluate the antioxidant activity of Quercetin in both *in-vitro* and in DEN-induced HCC in mice.

Materials and methods

Chemicals

Quercetin, DPPH, 30% H₂O₂, ascorbic acid, ammonium molybdate, potassium ferricyanide, Sucrose, Triton X-100, 5-5' dithiobis - (2- nitrobenzoic acid) [DTNB] and others chemicals were purchased from Sigma-Aldrich and HiMedia, India. All chemicals used in the study were of analytical grade.

Sample preparation

About 1 mg/ml stock solutions of Ascorbic acid and Quercetin were prepared in 0.1% DMSO solution.

DPPH radical scavenging assay

2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radicals scavenging assay was determined by the method (Mathangi and Prabhakaran, 2013) with slight modifications. Different concentration of Quercetin (10-50 µg/ml) was mixed with 1.5 ml of DPPH (1 mM, prepared in methanol). The reaction mixture was incubated for 30 min in the dark after which absorbance was measured at 517 nm. The radical scavenging capacity of Quercetin was then compared with ascorbic acid which is the standard reference compound. The percentage DPPH scavenging effect of Quercetin was then calculated using the following equation.

$$\% \text{ scavenging effect} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where A_{control} = absorbance of the control (which contains the entire reagent except the sample) and A_{sample} = absorbance of the test sample.

Hydrogen peroxide radical scavenging assay

This assay was performed according to the method described (Pavithra and Vadivukkarasi, 2014) with slight modification. Different concentrations of Quercetin (10-

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50 µg/ml) were mixed with 0.6 ml of hydrogen peroxide (40 mM) solution prepared in phosphate buffer (0.1 M pH 7.4). The reaction mixture was incubated for 10 min in dark and the absorbance was measured at 230 nm against a blank solution. Ascorbic acid was used as a standard reference compound. The percentage inhibition was then calculated using the following equation.

$$\% \text{ inhibition} = \frac{A \text{ control} - A \text{ sample}}{A \text{ control}} \times 100$$

Where *A control* = absorbance of the control (which contains all the reagents except the sample) *A sample* = absorbance of the test sample.

Reducing power assay (Ferric reducing activity)

Reducing power assay was performed according to the method (Hajaji *et al.*, 2010) with slight modification. Different concentrations of Quercetin (10-50 µg/ml) were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide [$K_3Fe(CN)_6$] (1%). The reaction mixture was incubated at 50 °C for 20 min after which 2.5 ml of trichloroacetic acid (10%) was added. The reaction mixtures were then centrifuged at 3000 rpm for 10 min. 2.5 ml was then taken from the reaction mixture and 2.5 ml distilled water was added, followed by the addition of 0.5 ml $FeCl_3$ (0.1%). This was allowed to stand for 10 min and the reaction mixture turned greenish. The absorbance was then measured at 700 nm against a blank solution. As concentration increases, absorbance increases which indicated an increase of reduction capability and this was compared with ascorbic acid.

Experimental animals

Swiss albino adult mice weighing 20-30g were obtained from the Pasteur Institute, Shillong, Meghalaya, India. The animal was grouped and housed in polyacrylic cages of 5 mice per cage and maintained standard laboratory conditions (temperature 25-28°C) with 12 h light and 12 h dark cycle. The experimental protocols were followed according to the Institutional Animal Ethical Committee (IAEC) regulations approved by the committee and conducted humanely.

Induction of Hepatocellular Carcinoma

The animals were divided into 3 groups of 5 each (I-III). The freshly prepared Diethylnitrosamine (DEN) (20 mg/kg BW) suspended in normal saline solution (0.1% DMSO) was administered by a single intravenously injection to induce hepatocellular cancer after overnight fasting for 18 h. Groups I served as a normal control received normal saline (0.1% DMSO) instead of Quercetin and DEN respectively, group II was treated with only DEN (20mg/kg BW) once a week for 6 weeks and group III was treated with DEN (20 mg/kg) once a week and Quercetin (50mg/kg) was administered intraperitoneally twice a week for 6 weeks.

Collection of sample

After 6 weeks, the animal was anesthetized and sacrificed, target organ such as liver was removed and washed with ice-cold saline and, weighed and cut into separate portions for antioxidant estimations, and the remaining tissues were stored immediately at -80 °C for future analysis.

Tissue analysis

Liver homogenate (1g) was prepared in a ratio of 1:10 (w/v) in ice-cold 0.25M sucrose (pH – 7.4) and homogenized by using a Teflon homogenizer. The homogenate was then centrifuged at 20,000g for 30 min at 4 °C. The supernatant was collected and stored at 4 °C, this supernatant was used for the estimation of catalase (CAT) (Aebi, 1984), Superoxide Dismutase (SOD) (Fridovich, 1986), Reduced Glutathione (GSH) (Owens and Belcher, 1964), and Acetylcholinesterase (AChE) (Oct *et al.*, 1975) and total protein concentration (Bradford, 1976).

Statistical analysis

All the determinations were conducted at least three times ($n = 3$); Linear regression analysis was used to calculate IC_{50} for both standard and Quercetin. The statistical analysis was carried out by Students' t-test, where P value < 0.05 was considered as statistically significant. Data were processed with graph pad prism version 8.02 software.

Results and discussions

DPPH radical scavenging assay

Quercetin was found to show DPPH free radical scavenging activity and was compared with ascorbic acid. The % inhibition of the various concentration of Quercetin as well as of ascorbic acid was calculated and a graph of concentration vs. % inhibition was plotted (Figure 1 and 2). The IC_{50} of Quercetin and ascorbic acid was calculated and was found to be 0.74 and 9.53, respectively as shown in table 1.

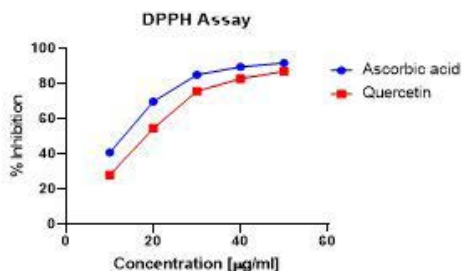


Figure 1. Percentage inhibition of Quercetin and Ascorbic acid at various concentrations on DPPH.

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Table 1. DPPH radical scavenging activities of ascorbic acid and Quercetin at different concentrations. Each value in the table is represented as mean \pm SD (n = 3).

Sample	Concentration ($\mu\text{g/ml}$)	% Inhibition (mean \pm SD)	IC ₅₀ ($\mu\text{g/ml}$)
Ascorbic acid	10	41.04 \pm 1.33	8.95
	20	70.02 \pm 1.76	
	30	85.28 \pm 0.55	
	40	89.83 \pm 0.37	
	50	92.06 \pm 0.14	
Quercetin	10	28.18 \pm 0.21	19.17
	20	54.79 \pm 0.16	
	30	75.96 \pm 0.12	
	40	83.07 \pm 0.25	
	50	87.28 \pm 0.15	

Based on their IC₅₀ values, it was found that ascorbic acid is a better radical scavenging compound than Quercetin. DPPH assay is the most commonly used method for screening antioxidant activity of various plant extracts or plant-based products as it is a very simple and sensitive method (Ilhami *et al.*, 2019). Using the assay, free radical scavenging properties of an antioxidant can be determined by measuring the decrease in absorbance of DPPH at 513 nm. The absorbance decreases when DPPH is being scavenged by an antioxidant through the donation of an electron or hydrogen. DPPH upon accepting an electron or hydrogen becomes a stable diamagnetic molecule with resulting changes in color from purple to slightly yellow.

Hydrogen peroxide radical scavenging assay

Quercetin was found to show H₂O₂ radical scavenging activity and was compared with ascorbic acid. The % inhibition of all the various concentrations of Quercetin as well as of ascorbic acid was calculated and a graph was plotted (Figure 3). The IC₅₀ of Quercetin and ascorbic acid was calculated from the graph and was found to be 16.26 and 36.22, respectively as shown in table 2.

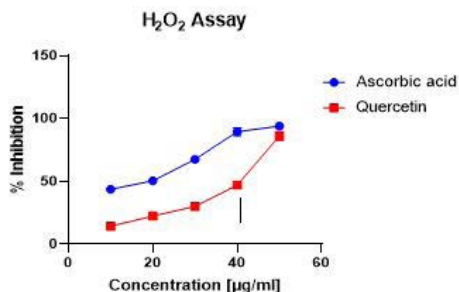


Figure 2. Percentage inhibition of Quercetin and Ascorbic acid at various concentrations on H₂O₂.

Table 2. H₂O₂ radical scavenging activities of ascorbic acid and Quercetin at various concentrations. Each value in the table is represented as mean ± SD (n = 3).

Sample	Concentration (µg/ml)	% Inhibition (mean ± SD)	IC ₅₀ (µg/ml)
Ascorbic acid	10	43.68 ± 1.43	16.26
	20	50.48 ± 2.38	
	30	67.55 ± 2.77	
	40	89.38 ± 3.02	
	50	91.97 ± 2.36	
Quercetin	10	14.36 ± 2.49	36.22
	20	19.49 ± 1.28	
	30	30.08 ± 0.77	
	40	46.92 ± 1.03	
	50	86.04 ± 3.54	

Hydrogen peroxide is a strong oxidizing agent which can oxidize several biomolecules and cause oxidative stress (Ilhami *et al.*, 2019). H₂O₂ itself is not very reactive, but it can sometimes be toxic to cells because it may give rise to hydroxyl radicals in the cells (Vadivukkarasi and Pavithra, 2014). In this assay, a decrease in the absorbance of H₂O₂, which absorbed maximally at 230 nm was observed as the concentration of antioxidants increases. This is because H₂O₂ is being scavenged by the compounds and higher the concentration of compounds, more is the scavenging effect and hence, lesser is

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the amount of H_2O_2 in the reaction mixture which resulted in decreasing absorbance. The IC_{50} of ascorbic acid and Quercetin are 16.26 and 36.22, respectively which indicates that ascorbic acid has a higher H_2O_2 scavenging capacity than Quercetin.

Reducing power assay (Iron reducing activity)

Reducing power assay of Quercetin was evaluated and compared with ascorbic acid and from the graph; we can see that Quercetin has less Fe^{3+} reducing capability compared to Ascorbic acid. The reductive capabilities were found to increase with increasing concentration of both ascorbic acid and Quercetin as shown in figure 3.

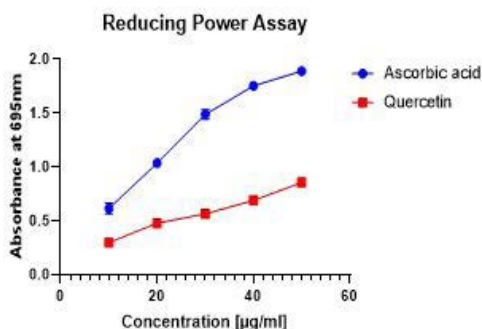


Figure 3. Reducing Capacity of Quercetin and Ascorbic acid at various concentrations.

This assay is based on the reduction of Fe^{3+} ions. The reducing capacity of ascorbic acid and Quercetin was compared and it was found that Ascorbic acid has higher reducing capability than Quercetin. This shows that Ascorbic acid can donate an electron very easily to Fe^{3+} . As we know that free radicals have free unpaired electron which makes it very reactive towards biomolecules, and therefore, the presence of a reductant, an electron donor, will stabilize the reactive radicals. Hence from this assay, we can predict the reducing power of antioxidant compounds or plant-based products based on their ability to reduced Fe^{3+} .

Liver oxidative stress markers

The antioxidant enzyme activities such as CAT, SOD, and GSH were found to be decreased significantly ($P \leq 0.05$) and AChE activity was found to be significant increases ($P \leq 0.05$) in DEN-treated mice when compared to normal control (Group: I) (Figure 4).

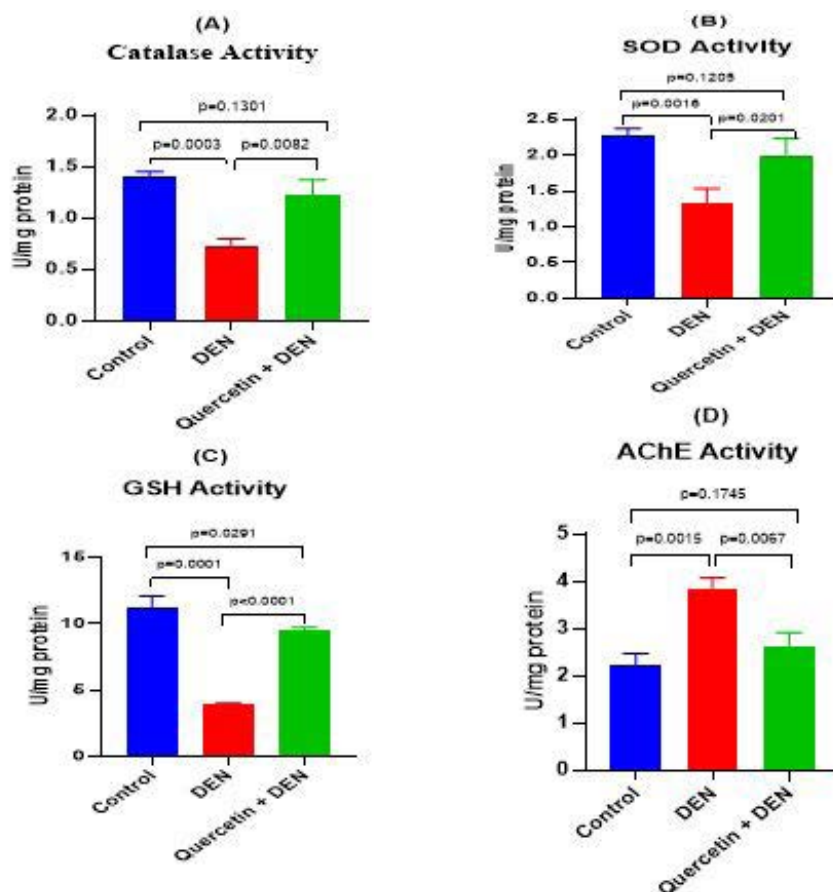


Figure 4: Effects of Quercetin on changes in liver enzyme levels of mice treated with DEN. (A) Catalase (CAT), (B) *Superoxide dismutase* (SOD), (C) Glutathione (GSH) (D) Acetylcholinesterase (AChE). I- Normal control, II –DEN treated (20mg/kg BW), III- Quercetin treated (50 mg/kg), + DEN (20mg/kg BW). Values are mean \pm S.D, error bar indicating the standard deviation, n = 5 animals. *p*-value less than 0.05 ($p \leq 0.05$) is statistically significant.

Catalase is a heme-containing enzyme that can protect the cells against oxidative stress caused by reactive oxygen species, such as H_2O_2 by degrading it into oxygen and water. Catalase activity was decreased significantly ($p=0.0003$) in DEN- treated mice (Group II) compared to the control group (Group I). Treatment with Quercetin (20mg/kg) significantly elevated ($p=0.0082$) when compared with Group II and showed no significant changes ($p=0.1301$) with the activity of the control group as shown in figure 4(A). The decrease in SOD activity ($p=0.006$) was observed in DEN-treated mice when compared with Group I, and significantly elevated ($p=0.020$) in activity compared with Group II, as close to the activity of Group I which show no significant changes ($p=0.1205$) as shown in figure 4 (B). Superoxide Dismutase (SOD) catalyzes the dismutation of superoxide to either ordinary molecular oxygen or to more stable compounds such as H_2O_2 which in turn degraded by catalase. Similarly, the reduced GSH decreased significantly ($p=0.0001$)

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in DEN-treated mice (Group II). Treatment with Quercetin (50mg/kg) significantly elevated ($p < 0.0001$) when compared with Group II, and the GSH activity is almost of the same level as that of control Group I as shown in figure 4 (C). GSH is required to maintain the normal reduced state and to counteract the deleterious effects of oxidative stress. During the reduction of hydrogen peroxide, GSH is oxidized to GSSG. When GSSG levels increased, the GSH-reductase activity was activated to convert GSSG in GSH (Cui *et al.*, 2011). In Group II, the low level was observed which indicates that GSH is being oxidized to GSSG as a result of increased radicals due to oxidative stress. Thus, GSH/GSSG homeostasis is disrupted. However, in Group III, even though the mice are exposed to DEN, its level is almost equal to that of the healthy mice. Thus, treatment with Quercetin showed a significantly elevated level of CAT, SOD, and CAT. This shows that Quercetin plays an important role in preventing oxidative stress by reducing the level of reactive oxygen species in a biological system.

AChE is an enzyme that catalyzes the breakdown of acetylcholine into choline and acetyl group and breakdown of many other choline esters that function as neurotransmitters. AChE is found mainly at neuromuscular junctions and in chemical synapses of the cholinergic type, where its activity serves to terminate synaptic transmission. The Acetylcholinesterase (AChE) activity was found to be significantly increased ($p = 0.0015$) in Group II, whereas its activity in Group III showed no significant changes ($p = 0.1745$) when compared with Group I as depicted in figure 4(D). Although AChE is well known for its function at cholinergic synapses, it also plays a non-catalytic role where it participates directly or indirectly in motility, proliferation, differentiation and cell-cell interaction. Studies have found that AChE function as a tumor growth suppressor in hepatocellular carcinoma and also in lung cancer (Perez-Aguilar *et al.*, 2015). The increase of cholinesterase activity in DEN-treated mice may enhance cholinergic signalling and contribute to tumor progression. In Group III, even though DEN was administered but treatment with Quercetin significantly reduced the activity of AChE as closed to that of normal mice. This showed that Quercetin has anticancer properties that prevent cancer growth in the liver by reducing the activity or level of AChE.

Conclusion

Despite many advances in cancer therapy, cancer is still one of the major causes of mortality worldwide. Natural products, such as Quercetin (3,3',4',5,7-pentahydroxyflavone), which is abundantly found in apples, red grapes, onions, raspberries, honey, cherries, citrus fruits, and green leafy vegetables, exerts various biological effects including antioxidant, anticancer, antiviral, etc. This current study demonstrated the antioxidant properties of Quercetin in both *in vitro* and *in vivo*. The *in vitro* analysis revealed that Quercetin is a good free radical scavenging species. Although ascorbic acid, a well-known antioxidant shows a much higher activity, Quercetin still shows a good free radical scavenging activity. To further confirm its antioxidant properties, an *in vivo* study was carried out and it was found that Quercetin significantly increases the activity of antioxidant enzymes (CAT & SOD) and GSH level and significantly reduces the liver marker enzyme (AChE) activity

when compared to DEN treated group. Thus, in each assay, Quercetin retained the activity of liver oxidative markers to about the normal level. From these observations, Quercetin shows excellent antioxidant and hepatoprotective properties, which might be useful for the treatment of oxidative-stress related diseases such as Hepatocellular carcinoma HCC.

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Atherosclerotic plaque development: Disease Pathogenesis and emerging treatment options

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Abstract

Atherosclerosis is a process of plaque formation and it manifest into different cardiovascular diseases. Basic mechanism that helps in Artherogenesis are Trans-migration of different granulocytes in the intimal layer of the artery with the help of adhesion molecules expressed in the outer layer of the activated endothelium. Excess circulatory Low Density Lipoprotein (LDL) enters the intimal layer via dysfunctional endothelium and gets oxidized into Oxidized-Low Density Lipoprotein (OX-LDL) and further entrapment of OX-LDL leads to macrophage to foam cell conversion on a gradual feeling on the OX-LDL in the intima. Foam cell formation leads to series of enzymatic Reactive Oxygen Species (ROS), scavenging receptors and chemokine's production and ultimately leading towards the oxidative pathways which end up in the inflammation and further complications leading towards several different cardiovascular diseases.

Keywords: Atherosclerosis, Plaque, Cardiovascular, Artherogenesis, Macrophage, Chemokine, inflammation.

Introduction

Atherosclerosis is a gateway to several cardiovascular diseases (CVDs) and it is a major cause of deaths throughout the globe (Murray and Lopez, 1997). By 2015 there were estimated 17.7 million deaths reported due to CVDs which corresponds to 31 % of all global deaths. Out of all the different CVDs major deaths were due to stroke (6.7 million deaths) and due to coronary heart disease (WHO CVDs report, 2017). Atherosclerosis means hardening and at the same time narrowing of the arteries and the major factor behind this is atherosclerotic plaque formation. Atherosclerotic plaques are basically deposits which are made up of cholesterol, fatty substances, cellular waste products, calcium and fibrin (a clotting material in the blood) (Zaman *et al.*, 2000). Coronary arteries supply oxygen to heart with the help of circulatory system. When there is a plaque buildup in the arterial wall it leads to less oxygen supply to the cardiac muscle which initially leads to vomiting, anxiety, angina, coughing and feeling faintness. On a regular interval of time due to the lack of oxygen supply to the cardiac muscle cell may lead to myocardial ischemia, which results in the myocardial cell death and ultimately give rise to cardiac arrest (Libby, 2002). Plaque built up in the carotid artery may give rise to less blood supply to the brain. It may causes weakness, dyspnea, headache, facial numbness, paralysis and it can turn out to be as lethal as stroke (Elkind, 2006). The coronary artery wall consists

of three layers. The inner layer is called intima, the middle layer is called media, and the outer one is denoted the adventitia (Davis *et al.*, 1988). According to the hypothesis of endothelial dysfunction, Elevated amount of circulatory LDL (Low Density Lipoprotein) in the lumen enters the intimal layer which is the middle layer in the arterial wall with the help of dysfunctional endothelial cells and it return the dysfunctional endothelial cells release several Reactive Oxygen Species (ROS) as well as several metalloprotease results in the oxidation of LDL to OX-LDL (Oxidized-LDL) which in turn leads to the activation of the endothelial cells (Incalza *et al.*, 2017). Activated endothelial cell express receptors/adhesion molecules for the white blood cells (WBC's) on their surface and this helps in the migration of monocytes and T-helper cells into the intimal layer (Schnoor *et al.*, 2015). After migration of the Monocytes into the intimal layer in get differentiated into macrophage and starts to feed on OX-LDL and gradual accumulation of OX-LDL gives rise to the foam cell formation. Foam cells significantly contribute in the pathogenicity of atherosclerosis in many different ways and the most important role involves the release of Insulin-like growth factor-1 (IGF-1) which helps in the migration of the smooth muscle cells (SMC's) from the tunica media to the intimal layer and its proliferation (Sukhanov *et al.*, 2015). Foam cells secrete pro-inflammatory cytokines such as interleukins: IL-1, IL-6; tumour necrosis factor (TNF); chemokines: chemokines ligand 2, CCL5, CXCL1; as well as macrophage retention factors to the lumen which helps attract more macrophages to enter the intimal layer (Fatkhullina *et al.*, 2016). High number of SMC's indirectly promotes the synthesis of collagen formation which in turn leads to the hardening of the atherosclerotic plaque itself (Rocnik *et al.*, 1998). High numbers of foam cells accumulation and its gradual death leads to high lipid content release. The foam cell debris involves the genetic material which majorly attracts neutrophil cells (Döring, Y. *et al.*, 2015). Simultaneously due to released lipids, pro-inflammatory cytokines and ROS which leads to inflammation in the plaque area of the artery. The vasa vasorum is a network of small blood vessels that increase the blood supply to the layer of the tunica intima (Xu *et al.*, 2015). Meanwhile the T-cells exploits the adhesion receptors present in the endothelial cells and get activated by the residing macrophages and release substances such as Interferon gamma (IFN γ) which enhances the level of inflammation and attracts more white blood cells by further activation of the endothelial cells (Moss and Ramji, 2015). As the atherosclerotic plaque grows bigger in size it generates tremendous pressure in the arterial walls which ultimately results in the plaque rupture which is also known as thrombosis (Schnoor, *et al.*, 2015). Thrombosis is a process in which the coagulation process gets activated to stop the plaque from spilling out into the lumen and gives rise to thrombus or a clot which restrains the normal blood circulation of the artery (Otsuka *et al.*, 2016).

Atherosclerosis risk factors and indicators

The exact causes and risk factors of atherosclerosis are unknown; however, certain conditions, traits, or habits may raise the chance of developing atherosclerosis. Most risk factors including high cholesterol and LDL, low level of high density lipoprotein (HDL)

in the blood, hypertension, tobacco smoke, diabetes mellitus, obesity, inactive lifestyle, Age can be controlled and atherosclerosis can be delayed or prevented. (Ross, 1993; Owen *et al.*, 2011; Weber *et al.*, 2011)

Cholesterol increase

Cholesterol is a hydrophilic lipid that is progenitor of steroid hormones such as corticosteroids, sex hormones, bile acids, and vitamin D. Cholesterol is one of the major component of cell membrane. Half of the body's cholesterol is provided by synthesis, mainly in the liver of mammals while all tissues containing nucleated cells are able to synthesize cholesterol. (Ohara *et al.*, 1995; Corsini *et al.*, 1996; Nasri, 2013).

Homocysteine

There is a substantial evidence to prove increased plasma homocysteine levels as a risk factor for atherosclerotic vascular disease (Mayer *et al.*, 1996). Homocysteine is a thiol-containing amino acid intermediate formed during the metabolism of methionine, an essential amino acid. In healthy persons, plasma homocysteine levels are between 5 and 15 $\mu\text{mol/L}$ in the fasting state (Ueland *et al.*, 1993). Increased homocysteine plasma levels have been associated with aging, (Kang *et al.*, 1986) menopause (Jacobsen *et al.*, 1994) chronic renal insufficiency (Chauveau *et al.*, 1993), low plasma levels of vitamin cofactors (B_6 , B_{12} , and folate) (Ubbink *et al.*, 1993) and cardiac transplantation (Berger *et al.*, 1995).

Impaired fibrinolysis

Fibrinolytic system consists of plasminogen, which is converted to its active form plasmin by plasminogen activators, including Tissue plasminogen activator (tPA). One of the Inhibitors of this system is plasminogen activator inhibitor type 1 (PAI-1) and plasmin inhibitor such as α_2 -antiplasmin. In the Northwick Park Heart Study, fibrinolytic activity was measured by dilute blood clot lysis time at study entry in 1382 men, age 40 to 64 years, of which 179 subsequently experienced episodes of CAD during a mean follow-up of 16.1 years (Meade *et al.*, 1993). In the men who were 40 to 54 years old, impaired fibrinolysis was associated with a significantly increased risk of CAD ($P=.002$), even after adjustment for plasma fibrinogen. No significant association was noted in older men (Ridker *et al.*, 1994).

Diabetes

Elevated glucose levels may contribute to the development of atherosclerosis in people with diabetes, independent of other risk factors (Selvin *et al.*, 2005). Atherosclerosis is the cause of a majority of cardiovascular events, and atherosclerosis is accelerated by diabetes and the metabolic syndrome. Many risk factors are associated with the metabolic syndrome and help explain the increased cardiovascular disease (CVD) in that condition (Reilly *et al.*, 2003). Because the metabolic syndrome occurs in most people with type-2 diabetes, its presence likely accounts for most of the increased incidence of CVD in type-

2 diabetes (Alexander *et al.*, 2003). However, the presence of diabetes increases the risk of CVD beyond that seen with the metabolic syndrome alone (Alexander *et al.*, 2003). Moreover, CVD risk is increased in type 1 diabetes (Dorman *et al.*, 1984), in which the presence of the metabolic syndrome and these other risk factors is less common. Type-1 diabetes (T1DM) and obesity are main risk factors for cardiovascular events (Burke *et al.*, 2008; Margeirsdottir *et al.*, 2008). In particular, young adults with T1DM have an increased risk of early asymptomatic atherosclerosis and consequent cardiovascular morbidity and mortality (Jørgensen *et al.*, 2005; Larsen *et al.*, 2005; Orchard *et al.*, 2006). Similarly, childhood obesity has been reported associated with biochemical and inflammatory factors that affect vascular endothelial function and that might confer a premature atherogenicity (Aggoun *et al.*, 2007). Moreover, the insulin resistance, key feature of obesity, metabolic syndrome and type-2 diabetes, results in an array of metabolic and vascular events which finally promote the development of atherosclerosis (Cubbon *et al.*, 2009).

Hypertension

Hypertension is a major risk factor in cardiovascular diseases and stroke. These complications are generally caused by high diastolic blood pressure. Hypertension damages endothelium by increasing the hemodynamic pressure on endothelium and may increase the permeability of arterial walls for lipoproteins. Elevated angiotensin II concentration stimulates SMC growth, increases inflammation and finally accelerates LDL oxidation in such patients. (Asgary *et al.*, 2013; Asgary *et al.*, 2014). Arterial chronic hypertension (HTN) is one of the established cardiovascular risk factors for development of atherosclerosis (Dzau, 1990) and an increased incidence of peripheral vascular disease (Murabito *et al.*, 1997), cerebrovascular disease (Lewington *et al.*, 2002). Although, the complications of hypertension were formerly attributed to diastolic blood pressure, there is much evidence showing that systolic blood pressure plays a role as well.

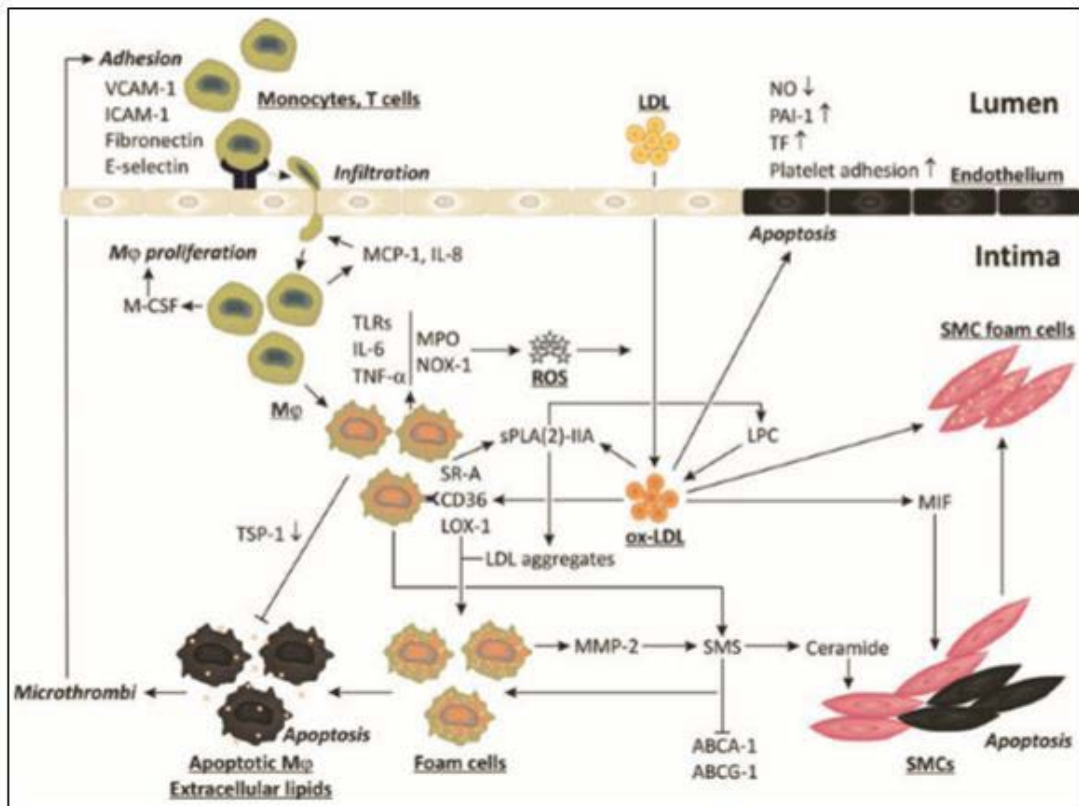


Figure 1: Molecular mechanisms involved in both oxidative as well as inflammatory pathways in the atherogenesis. (Picture courtesy: (Hulsmans and Holvoet, 2010))

The mechanism with which hypertension can accelerate atherosclerosis is still unknown; however, in animals fed with high fat, hypertension accumulates the fatty substances inside the arterial walls. (Khajehdehi *et al.*, 2012; Asgary *et al.*, 2013; Madihi *et al.*, 2013; Asgary *et al.*, 2014; Hajivandi, 2014).

Molecular mechanisms of oxidative stress in atherosclerotic plaques

Activated endothelial cells express adhesion molecules such as ICAM-1, VCAM-1, E-selectin and fibronectin leads to transcytosis and activation of inflammatory cells in to the intimal layer and in turn it helps in the expression of enzymes such as Myeloperoxidase, xanthine oxidase, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and nitric oxide synthase (Burtenshaw *et al.*, 2019). The consequence for this results in the production of ROS which in turn oxidises the phospholipids and the LDL into OX-LDL and its entrapment into the intimal layer. This sudden outburst of oxidative stress leads to further expression of chemokine's such as Monocyte chemo attractant protein-1 and Interleukin 8 which attracts primarily neutrophils and other granulocytes (Hulsmans and Holvoet, 2010). The existing macrophage in the intimal layer release macrophage colony-stimulating factor and help in its proliferation and simultaneously

express the scavenging receptors such as cluster of differentiation 36 (CD36), Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and macrophage scavenger receptor (SR-A). These receptors help in the further accumulation of the OX-LDL & cholesterol by the macrophage and get converted to foam cells, finally resulting in apoptotic macrophages and exposure of thrombogenic lipids. Deficient TSP-1 expression is associated with a decreased phagocytosis of dead cells. Matrix metalloproteinases (MMPs) & Spingomyelinases (SMS) are expressed by the foam cells induces cell death of the smooth muscle cells by excess production of Ceramide (Bioactive lipid molecule). ATP-binding cassette transporter (ABCA-1) & ATP binding cassette subfamily G member 1 function get altered by the SMS results in the impairment in the cholesterol and lipid efflux from foam cells. Sheer pressure at the site of plaque formation also promotes the production and release of ROS (Leopold and Loscalzo, 2008). Oxidative stress in humans with coronary artery disease is also exacerbated by a reduction of vascular extracellular superoxide dismutase, normally an important protective enzyme against the superoxide anion (Madamanchi *et al.*, 2005).

Role of inflammation in atherosclerosis

Inflammation works in all the different stages of atherosclerotic plaque development and rapture. Ox-LDL induces TLRs of which the ligands enhance the expression of inflammatory mediators IL-6 and TNF-. Inflammation has its significant role in giving rise to a vulnerable plaque from its stable form by weakening the atherosclerotic fibrous cap and allowing the plaque to rapture quickly (Stefanadis *et al.*, 2017).

Table 1: Critical Enzymes involved in pathogenesis of atherosclerosis

Sl#	Gene name	Enzyme name	Pathogenecity in atherosclerosis	References
1	<i>MMP3</i>	matrix metallopeptidase 3	K/O-Restricts VSMC migration	(Johnson <i>et al.</i> , 2016)
2	<i>MMP9</i>	matrix metallopeptidase 9	k/O- Restricts VSMC migration and plaque rapture	(Johnson <i>et al.</i> , 2016)
3	<i>PLAU</i>	plasminogen activator, urokinase	activate plasmin & MMP9	(Johnson <i>et al.</i> , 2016)
4	<i>NOS3</i>	Nitric Oxide Synthase 3	oxidative stress in vascular wall by NO	(Channon <i>et al.</i> , 2000)
5	<i>MAPK1</i>	Mitogen-activated protein kinase 1	p38 MAPK pathway activation: oxidative stress & Inflammation	(Reustle and Torzewski, 2018)
6	<i>PONI</i>	Paraoxonase 1	Reduce macrophage related oxidative stress	(Farid and Horii, 2012)
7	<i>MTHFR</i>	methylenetetrahydrofolate reductase	677C-T mutation MTHFR is related to plasma total homocysteine levels	(Verhoef <i>et al.</i> , 1997)
8	<i>mTORC1</i>	mechanistic target of Rapamycin kinase C1	Inhibition leads to reduced chemokines & HIF 1 α production	(Kurdi <i>et al.</i> , 2015)
9	<i>iNOS</i>	Inducible NO synthase	Inhibition leads to reduced inflammation	(Detmers <i>et al.</i> , 2000)
10	<i>AKT1</i>	AKT serine/threonine kinase 1	loss of AKT1 reduces eNOS phosphorylation, Nitric oxide release, VSMC migration	(Hernando <i>et al.</i> , 2007)
11	<i>MPO</i>	Myeloperoxidase	induces endothelial dysfunction, LDL oxidation to OX-LDL	(Kamanna <i>et al.</i> , 2013)
12	<i>PARP1</i>	Poly(ADP-ribose) polymerase 1	Pharmacological inhibition leads to reduction in AS development	(Xu <i>et al.</i> , 2014)
13	<i>SOD2</i>	Superoxide Dimutase 2	Protect from oxidative stress in AS	(Vendrov <i>et al.</i> , 2017)
14	<i>DPP4</i>	Dipeptidylpeptidase-4	Inhibition of DPP4 reduces atherosclerosis and CVD risk	(Aroor <i>et al.</i> , 2018)
15	<i>SRC</i>	Src Tyrosine Kinase	lipid oxidation and AS	(Reddy <i>et al.</i> , 2009)
Abbreviations : K/O- knockout for the gene, AS- Atherosclerosis , CVD- Cardiovascular Disease, VSMC- Vascular Smooth Muscle Cells,				

Ox-LDL induces migration inhibitory factor that stimulates SMC migration. The uptake of ox-LDL by SMCs leads to the production of SMC foam cells and secretion of MMPs that degrade the extracellular matrix proteins rendering the plaque more prone to

rupture. OxLDL stimulates platelet adhesion and aggregation by decreasing endothelial production of nitric oxide, and enhances the pro-coagulant activity of endothelium by inducing the release of tissue factor. Ox-LDL reduces the fibrinolytic activity of endothelium by increasing the release of plasminogen activator inhibitor-1. Finally, ox-LDL induces apoptosis in endothelial cells (black) contributing to plaque erosion and rupture (Hulsmans and Holvoet, 2010). Lipid-laden macrophages in atherosclerotic plaques express MMPs. various extracellular stimuli, including reactive oxygen species, plasmin, and thrombin, contribute to enzymatic activation of these enzymes that is required for their proteolytic activity (Libby, 2013).

Key players involved in both oxidative stress & Inflammation in atherosclerosis

Oxidative stress and inflammation are the key factor in orchestrating atherosclerosis and ultimately leads its way to cardiovascular complications. *National Center for Biotechnology Information* (NCBI) is one of the largest sources for biological information. 1142 genes were considered which are involved in atherosclerosis and have a significant role in inflammation (www.ncbi.nlm.nih.gov/gene/?term=atherosclerosis+and+inflammation) similarly for oxidative stress and its role in atherosclerosis 672 genes were considered (www.ncbi.nlm.nih.gov/gene/?term=atherosclerosis+and+oxidative+stress). Out of which there are 444 genes which work in both atherosclerosis as well as inflammation (Figure 2). Among the common link between both atherosclerosis as well as inflammation there are several regulatory protein, proteins involved in different stress pathways, wound healing, immune responses, and have several different other biological functions but out of the of them 125 major enzymes that play significant role in the pathogenicity process were found. Either up regulation or down regulation of these enzymes may lead to significant change in the disease state, hence these can be potential target for future drugs development for CVD. Out of those 125 enzymes the most significant ones are depicted in the Table 1.

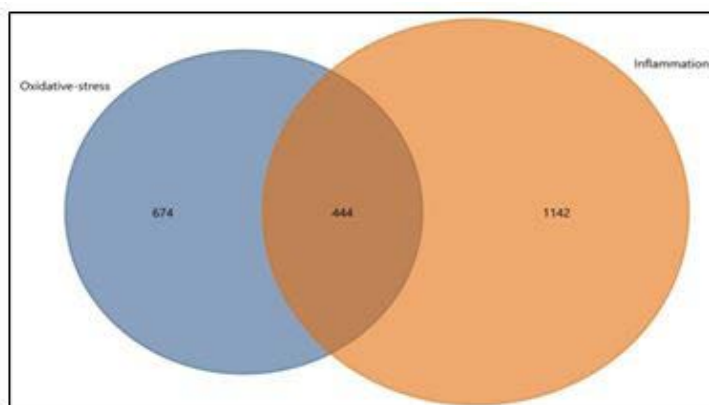


Figure 2: Venn diagram for the set of genes which are involved in oxidative stress & atherosclerosis in blue (674) and the set of genes involved in inflammation & atherosclerosis are in orange (1142) and the intersection of genes (common one's) which are involved in both oxidative stress as well as inflammation (444).

Treatment options and emerging therapy for atherosclerosis

Among the established therapies, the use of Statins and Astrovastatin leads to primary and secondary control of the disease through inhibition of cholesterol synthesis and acting as an anti-inflammatory agent. Aspirin, clopidogrel, prasugrel and ticagrelor inhibits platelet aggregation, thereby controlling the secondary progression of the disease. Among the emerging therapies, HDL mimetics, for example, apoA1-Milano, promotes cholesterol efflux and acts as an anti-inflammatory agent. The drugs are in Clinical phase 1 and 2 trials. Methotrexate acts as an immunosuppressive and this drug is currently under Clinical phase 3 trials. Again, there are a number of novel therapies that are showing promise, for example blocking the CD40-TRAF6 interaction site limits the atherosclerosis of unstable phenotype in mice. CCL17 inhibition interestingly attenuates lesion formation in mice (Table 2).

Table: 2: Treatment options for atherosclerosis and their outcome

Compound or Method	Mechanism Involved	Outcome	References
Established Therapies			
Statins, for example, atorvastatin and rosuvastatin	Inhibit cholesterol synthesis, anti-inflammatory	Primary and secondary prevention	Ray and Cannon (2005); Nissen (2005) and Ridker <i>et al.</i> (2009)
Nicotinic acid (niacin)	Inhibits fat breakdown in adipose tissue and increases HDL cholesterol, anti-inflammatory	Secondary prevention	Taylor <i>et al.</i> (2009); Lukasova (2011)
Aspirin, clopidogrel, prasugrel, ticagrelor	Inhibit platelet aggregation	Secondary prevention	Wallentin <i>et al.</i> (2009); von Hundelshausen and Weber (2007)
β-blockers	Antihypertensive	Secondary prevention	Sipahi <i>et al.</i> (2007)
Renin-angiotensin system inhibitors	Antihypertensive	Secondary prevention	Yusuf <i>et al.</i> (2008)
Emerging Therapeutic approaches			
HDL mimetics, for example, apoA1-Milano	Promote cholesterol efflux, anti-inflammatory	Clinical phase 1 and 2	Nissen <i>et al.</i> (2003); Navab <i>et al.</i> (2010)
Darapladib (selective Lp-PLA2 inhibitor)	Decreases atherogenic lipid production	Clinical phase 3	Wilensky <i>et al.</i> (2008); Wilensky <i>et al.</i> (2008)
IL-1ra (IL-1 receptor antagonist)		Clinical phase 2	Klingenberg and Hansson (2009)
Methotrexate	Immunosuppressive	Clinical phase 3	Klingenberg and Hansson (2009)

Novel experimental Strategies			
Blocking the CD40-TRAF6 interaction site	Impaired recruitment of Ly6C ⁺ monocytes to the arterial wall, and polarization of macrophages toward an antiinflammatory regulatory M2 signature	Limits atherosclerosis of unstable phenotype in mice	Lutgens <i>et al.</i> (2010)
Blocking MIF receptor binding	Impaired monocyte adhesion to the arterial wall in atherosclerosis-prone mice	Induces lesion stabilization and regression in mice	Bernhagen <i>et al.</i> (2007); Weber <i>et al.</i> (2008); Kraemer <i>et al.</i> (2011)
Maraviroc (CCR5 antagonist)	Blocking CCR5	Approved for US and European markets for HIV treatment	Koenen and Weber (2010)
MLN1202 (CCR2-specific antibody)	Blocking CCR2	Clinical phase 2	Gilbert <i>et al.</i> (2011)
Nonagonistic CCL2-competing mutant PA508	Nonagonistic plus increased proteoglycan Affinity	Attenuates lesion formation in mice	Liehn <i>et al.</i> (2010)
Dominant-negative CCL5 mutant [44AANA47]	Creates dimers devoid of proteoglycan binding	Attenuates lesion formation in mice	Braunersreuther <i>et al.</i> (2008)
Mkey (ct-2009)	Disrupts CCL5-CXCL4 heteromerization	Attenuates lesion formation in mice	Koenen, <i>et al.</i> (2009)
Immunization	Protective antibody generation, Treg cell induction	Can attenuate lesion formation in mice	Ludewig <i>et al.</i> (2000); Johansson <i>et al.</i> (2010); Habets <i>et al.</i> (2010); Hermansson <i>et al.</i> (2011); Klingenberg <i>et al.</i> (2010)
CCL17 inhibition	Supports Treg cell homeostasis	Supports Treg cell homeostasis	Weber <i>et al.</i> (2011)

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Okra- an important vegetable crop of India

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Abstract

Abelmoschus esculentus, commonly known as Okra, is a major vegetable crop of India. It is very popular among farmers and consumers because of its diverse usage as fiber yielding and ornamentals for their beautiful flowers. It is distributed in tropics, all over the world with several local variant genotypes. While *A. esculentus* show less genetic variability, it has genomic similarities identical with those of diploid *A. tuberculatus* and allopolyploid *A. tetraphyllus* var. *tetraphyllus* respectively. This homology confirms the lineage of *A. esculentus* from either of the two taxa.

Keywords: *Abelmoschus esculentus*, origin, distribution, diversity, phylogeny

Introduction

Okra [*Abelmoschus esculentus* (L.) Moench], also known as *Hibiscus esculentus* L. is a member of the mallow (Malvaceae) family and can be found as an annual (primarily the U.S.) or as a perennial in India and Africa (Lamont, 1999). Varying chromosome numbers (about $n=36$; 33; 59 to 72) have been reported for *A. esculentus* (Joshi et al. 1974). *A. esculentus* L. (Moench) is an economically important annual vegetable grown from seed, in tropical, subtropical and Mediterranean climatic zones. In West and Central Africa it is cultivated, in association with *A. caillei* where the former, which flowers earlier, is known as 'the rainy season okra' and the latter, which has a longer cycle (up to 1 year) is known as the 'dry season okra'. This crop is suitable for cultivation as a garden crop as well as on large commercial farms. It is grown commercially in India, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Burma, Japan, Malaysia, Brazil, Ghana, Ethiopian, Cyprus and the Southern United States. India ranks first in the world with 3.5 million tonnes (70% of the total world production) of okra produced from over 0.35 million ha land (FAOSTAT, 2008). Okra is known by many local names in different parts of the world. It is called lady's finger in England, gumbo in the United States of America, *guino-gombo* in Spanish, *guibeiro* in Portuguese and bhindi in India. It is quite popular in India because of easy cultivation, dependable yield and adaptability to varying moisture conditions. Even within India, different names have been given in different regional languages (Chauhan, 1972).

Origin and Distribution

A. esculentus is cultivated as a vegetable in most tropical and subtropical regions of Africa, India and America. In West Africa, Siemonsma (1982b) has clearly demonstrated that the species has preference for the Sudano-Sahellian zone. However, *A. esculentus* is also found in forest regions in smaller quantities. It is a case of ecological adaptation to photoperiodic response and to parasitism (different in savannah and forest areas). But in this Guinean bioclimatic zone, Siemonsma (1982a) has given prominence to a new cultivated species provisionally called “Guinean” okra, which can be found in the forest regions of Ghana, Guinea, Ivory Coast, Liberia and Nigeria.

A. esculentus is found all around the world from Mediterranean to equatorial areas. Cultivated and wild species clearly show overlapping in Southeast Asia, which is considered as the centre of diversity. The spread of the other species is the result of their introduction to America and Africa. There are two hypotheses concerning the geographical origin of *A. esculentus*. Some authors argue that one putative ancestor (*A. tuberculatus*) is native to Uttar Pradesh in northern India, suggesting that the species originated from this geographic area. Others, on the basis of ancient cultivation in East Africa and the presence of the other putative ancestor (*A. ficulneus*), suggest that the area of domestication is north Egypt or Ethiopia, but no definitive proof is available today. For *A. caillei*, only found in West Africa, it is difficult to suggest an origin outside. Its origin by hybridization with *A. manihot* is difficult to accept even if its presence, mentioned in the Flora of West Africa (Hutchinson and Dalziel, 1958) was not recently confirmed in this area and herbarium samples are lacking.

Uses

Okra is cultivated for its fibrous fruits or pods containing round, white seeds. The fruits are harvested when immature and eaten as a vegetable. The young fruits produced by this species are used as a vegetable. They are picked when still immature (3-6 cm long), before the differentiation of fibers and before the seeds are fully developed. After cooking, these young fruits have a mucous consistency; the water in which they are boiled becomes thick and ropy. They are consumed alone or in salad, after cooking in salty water, and are used in the preparation of certain African sauces. For year-round consumption, the fruits are conserved either in the form of slices dried naturally in the sun (Africa and India) or frozen or sterilized (USA). The leaves are also eaten as spinach by Africans. The fibers extracted from the stems are sometimes used, as on the banks of the Niger in Mali, to make strings and nets (Chevalier, 1940).

The roots and stems of okra are used for cleaning the cane juice from which gur or brown sugar is prepared (Chauhan, 1972). Its ripe seeds are roasted, ground and used as a substitute for coffee in some countries. Mature fruits and stems containing crude fiber are used in the paper industry. Extracts from the seeds of the okra is viewed as alternative source for edible oil. The greenish yellow edible oil has a pleasant taste and odour, and is

high in unsaturated fats such as oleic acid and linoleic acid. The oil content of the seed is quite high at about 40%.

Okra provides an important source of vitamins, calcium, potassium and other mineral matters, which are often lacking in the diet of developing countries (IBPGR, 1990). Okra pods are used in Asia as a vegetable, food ingredient, as well as a traditional medicine for many different purposes; for example, as diuretic agent, for treatment of dental diseases and to reduce/prevent gastric irritations. The healthy properties are suggested to originate from the high polysaccharide content of okra pods, resulting in a highly viscous solution with a slimy appearance when okra is extracted with water

Genetic Diversity

From careful perusal of published data, it is known that Indian representative taxa of *Abelmoschus*, including *A. esculentus*, indicate that the taxonomic units of *Abelmoschus* are heterogeneous in nature thereby exhibiting deviant chromosome numbers. The occurrence of different somatic chromosome numbers ranging from $2n=58$ to $2n=200$ in the genus suggest that the classification at the species level is much more complex than elaborated by van Borssum Waalkes (1966). Therefore, it is proposed to undertake investigations with a prime objective of a comprehensive study to understand the quantum of genetic diversity and ascertain genomic relationships among species of *Abelmoschus*, including *A. esculentus* which are important to systematists, evolutionary biologists, cytogeneticists, molecular biologists and plant breeders as well. The study is expected to find solutions to overcome the taxonomic dilemma that exists in the genus using cytogenetical and molecular approaches in both the cultivated and wild relative species from the Indian sub-continent. Thus, an attempt has been made to analyze the existing genetic variation among these plants in natural habitat to assess the quantum and range, and also to define the genetic basis for the same.

DNA markers for diversity analysis

Molecular marker-based approaches have been successful in characterizing variations both at inter- and intra-specific levels since they generate new genetic diversity parameters to define. Such parameters often extend beyond species boundaries and boast of a great value and significance in diagnostic applications. In the present study, three single primer amplification methods viz., random amplified polymorphic DNA (RAPD), inter-simple sequence repeats (ISSR) and directed amplification of minisatellite DNA regions (DAMD) were utilized (individually and cumulatively) to comprehend the genetic variation that exist both at intra- and inter-specific levels in cultivated and wild species of *Abelmoschus* taxa.

RAPD markers were able to easily distinguish the differences and similarities among 43 genotypes based on their randomness in their genetic make-up (as found in chromosome counts also) by clustering them into four distinct clusters, each cluster representing the respective species. Only one accession belonging to *A. tetraphyllus* var.

tetraphyllus (AT-2) behaved as an out-group, which can be visibly noted, not only from its gel profile but also from its collection site i.e. Nepal. *A. angulosus* var. *grandiflorus* shown to have close affinity towards *A. tetraphyllus* var. *tetraphyllus* which is evident from the successful F1 hybrids from this cross. *A. esculentus* with a stable chromosome number, $2n=130$ is considered low in genetic diversity and genetic similarity individuals in a given population. On closer analysis it is confirmed that RAPD analysis of *A. esculentus*, the cultivated taxon Pusa Sawani, AE-39 and AE-41 had shown the genetic similarity of 100% among genotypes in the population.

When species relationships were probed, *A. esculentus* showed close affinity towards *A. tetraphyllus* and *A. angulosus* var. *grandiflorus*, while *A. moschatus* ssp. *moschatus* has been clustered separately from the rest. DAMD marker also revealed high polymorphism across the 43 genotypes of the Indian representative *Abelmoschus* belonging to four species. *A. angulosus* var. *grandiflorus* showed the close proximity towards *A. tetraphyllus* by clustering together, while *A. esculentus* was distantly related from *A. tetraphyllus*. *A. moschatus* ssp. *moschatus* was closely related to *A. esculentus*.

Phylogenetic Analysis

The molecular phylogenetic analysis of various *Abelmoschus* taxa has been carried out using DNA sequence data of three chloroplast loci viz., *accD*, *atpB* and *psbK-psbI* which revealed different topologies in all the four tree building methods. The clustering pattern of *accD* and *atpB* sequences were almost similar, though with some exceptions in the positions of the species may differ in the tree. It was recorded that *atpB* had the highest percentage of conserved sites while the highest percentage of variable sites were recorded in intergenic spacer, *psbK-psbI*. The lowest percentage of sequence divergence substitution was recorded as 2.19 in *atpB*, confirming that nucleotide substitutions occur at a relatively slow rate in cpDNA (Curtis and Clegg, 1984). These sequence comparisons have further revealed that, although the genome as a whole change slowly, certain genes change either more rapidly or more slowly than the average. The region is highly conserved in *Abelmoschus* and relatively few sites in the aligned data matrix are parsimony informative, a variety of relationships among the species are revealed by the analyses, some of which are congruent with the known species relationships.

In the present study, the coding gene *accD* and *atpB*, and intergenic spacer region *psbK-psbI* has been combined into a single matrix for a collective approach analysis to obtain greater phylogenetic resolution. The concatenate topologies of the trees constructed by ML, MP, BI and NJ for the consensus sequence were consistent with the clustering of *A. caillei* and *A. tetraphyllus* var. *tetraphyllus*, in all the methods, thereby it supports our earlier hypothesis that *A. tetraphyllus* var. *tetraphyllus* may be one of the probable progenitor species of *A. caillei*. Since cpDNA is maternally inherited, one can conclude that *A. esculentus* and *A. caillei* were essentially identical with those of diploid *A. tuberculatus* and allopolyploid, *A. tetraphyllus* var. *tetraphyllus* respectively, thereby either of the latter two species served as the maternal parents in the inter-specific hybridizations which gave

rise to the hybrid taxa. The origin of amphidiploid, *A. esculentus* is further supported from the inter-specific hybridization studies.

Conclusion

Okra (*A. esculentus*), is an important vegetable crop of India and it has many other uses, for eg. fibres extracted from the stems are sometimes used to make strings and nets. *A. esculentus*, an amphidiploid in origin, is an interspecific hybrid and exhibit low genetic diversity within the population. *A. esculentus* is identical with those of diploid *A. tuberculatus* and allopolyploid *A. tetraphyllus* var. *tetraphyllus* respectively, thereby either of the latter two species served as the maternal parents in the inter-specific hybridizations which gave rise to the hybrid *A. esculentus*.

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A preliminary note on big data and machine learning technologies

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Abstract

Big data is high-volume, high-velocity and variable information assets which demand cost-effective, innovative forms of processing for enhanced insight and decision making. An essential quality of the Big Data is the large volume which is heterogeneous and of different dimensions. Data mining and machine learning systems are utilized to separate the important and concealed examples from the huge volume of data. Many machine learning strategies are coordinated with big data analytics tools.

Keywords: Machine learning, Big data, Data mining.

Introduction

In recent times there has been an exponential production of data from various sources of the web, smart phones or smart sensors, which has lead to generation of big data. The term big data can be referred to as enormous, fast, arising, various classes and with parts of undesirable noises that are hard to store, process, analyze, translate, expend and settle for better decision in the field of medicinal services, funds, and business or industries. Gigantic data have originated from people through the usage of PC, advanced mobile phones, gadgets which are utilized to share message and recordings with companions in internet based life such as Facebook, Instagram, Whatsapp, etc, for sharing short clips, share their perspectives and purchase where data gathering has developed enormously and is already past the capacity of commonly utilized software tools to capture, manage, and process inside a “tolerable elapsed time” (Wu *et al.*, 2014) (Blazquez and Domenech, 2018)

Indeed, the activity of people and their exercises are recorded by smart sensors which are set in part of urban communities and in diverse public places. The most fundamental challenge for Big Data applications is to investigate the enormous volumes of data and focus on helpful data or information for future activities. In many cases, the learning/extraction procedure must be productive and near continuous on the grounds that putting away all watched data(Wu *et al.*, 2014).

Big Data begins with huge volume of heterogeneous, self-ruling sources with distributed and decentralized control, and tries to investigate complex and advancing connections among data which is known as HACE Hypothesis. These attributes make

it an extraordinary test for helpful learning from Big Data (Wu *et al.*, 2014, Chen and Zhang, 2014, Ke and Shi, 2014.)

Big Data: Big data is a term for data sets that are so large or complex that traditional data processing application software is inadequate to deal with them. Big data challenges include capturing data, data storage, data analysis, search, sharing, transfer, visualization, querying, and updating and information privacy (Saidulu *et al.*, 2017). Big data is high-volume, high-velocity and high-variety information assets that demand cost-effective, innovative forms of information processing for enhanced insight and decision making. (Khine and Shun, 2017)

One of the essential qualities of the Big Data is the large volume of data which is heterogeneous and of different dimensions. Distinctive data gatherers lean toward their very own schemata or protocols for data recording, and the idea of various applications brings about varying data representations (Wu *et al.*, 2014, Ke and Shi, 2014).

However, there are traditional techniques for managing huge data and learning from these enormous data, open doors for Machine learning (ML). It is a sort of artificial strategy which is utilized for finding information from enormous data for settling on better astute decisions (Saidulu *et al.*, 2017). Machine learning algorithms arrange the learning task in three classes, *viz.* supervised, unsupervised and reinforcement learning. (Lakshmi and Sheshasaayee, 2015, Sheshasaayee and Lakshmi, 2017.)

Machine Learning: Machine Learning is a plan to gain from models and experience, without being expressly customized. Rather than composing code, one can feed data to the conventional algorithm, and it constructs relation dependent on the data given (<https://towardsdatascience.com/introduction-to-machine-learning-db7c668822c4>).

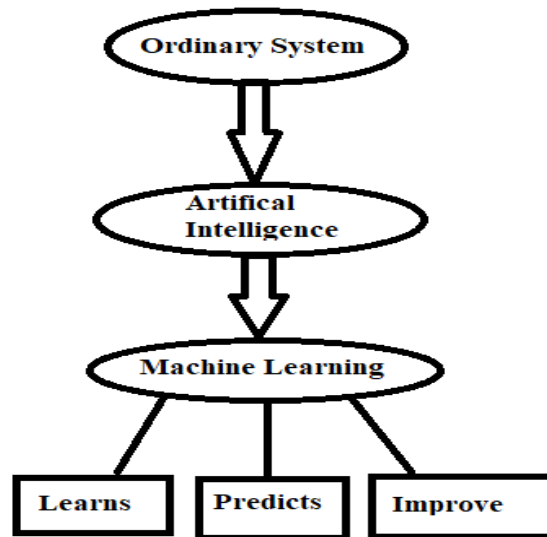
Machine learning is a data analytics procedure that instructs PCs to do what falls into place without any issues for people and creatures: gain from experience. Machine learning algorithms utilize computational techniques to “learn” information legitimately from data without depending on a predetermined condition as a model. The algorithms adaptively improve their exhibition as the quantity of tests accessible for learning increments. Deep learning is a specific type of machine learning (<https://in.mathworks.com/discovery/machinelearning.html>).

Machine Learning is a field which is raised out of Artificial Intelligence (AI). Applying Artificial Intelligence, we needed to manufacture better and keen machines. (Khine and Shun, 2017) In any case, besides a couple of unimportant undertakings, we were not able to program the unpredictable and always encounter difficulties. Therefore, the best way to have the option to accomplish such undertaking is to give machine a chance and gain from itself.

Machine learning, in present times is available in such a significant number of fragments of innovation that we don’t understand while utilizing it (<https://towardsdatascience.com>).

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Example:



As an example, it can be assumed that a system is fed with input data that contains the photographs of the students of a college. At that point we do:

- Analysis of data. The system attempts to discover patterns, for example tallness, size, etc.
- Dependent on these patterns, the system attempts to predict various types of student have a place with specifics course, fittest student, etc and partition them.
- Finally, it keeps all tracks of the choices; to ensure that it is learning. At this point of time when requested, the machine can predict and isolate the various kinds of students. Thus, the machine does not repeat the whole procedure once more. That is how the machine learning works.

With the rise in big data, machine learning has become a key technique for solving problems in diverse areas, such as: **Automotive, aerospace, and manufacturing**, for predictive maintenance, **computational biology**, for tumor detection, drug discovery and DNA sequencing, **computational finance**, for credit scoring and algorithmic trading, **energy production**, for price and load forecasting, **image processing and computer vision**, for face recognition, motion detection, and object detection, **natural language processing**, for voice recognition applications. Generally, the field of machine learning is divided into three sub domains: Supervised learning, unsupervised learning, reinforcement learning (Mustafi, 2016)

Supervised Learning: The machine learns from the training data that is labeled. So one has to supervise the machine learning while training it to work on its own. Supervised

learning requires training with labeled data, which has inputs and desired outputs.

Unsupervised Learning: The machine learns from the training data but without label. Unsupervised learning finds hidden patterns or intrinsic structures in data. It draws inferences from data sets consisting of input data without labeled responses (<https://in.mathworks.com/discovery/machinelearning.html>).

Reinforcement Learning: The machine learns on its own i.e. by its mistake and experiences. (Chen and Zhang, 2014)

Over the last few decades, heart disease has been the most common cause of casualties around the globe. An early detection of heart disease and continuous monitoring can reduce the mortality rate. The exponential growth of data from different sources such as wearable sensor devices used in health monitoring activities- thanks to Internet of Things. This resulted in generation of an enormous amount of data on continuous basis. The combination of streaming big data analysis and machine learning is a breakthrough technology that can have a significant impact in healthcare especially for an early detection of disease, so developing a distributed and real-time healthcare analytics system using traditional analytical tools is extremely complex, while exploiting open source big data technologies can do it in a simpler and more effective way. A real time heart disease prediction system based on apache Spark which stands as an effective large scale distributed computing platform which can be used successfully for streaming data event against machine learning through in-memory computations has been recently developed. (Sheshasaayee and Lakshmi, 2017, Lakshmi and Sheshasaayee, 2015).

One important developmental discovery made by software engineers is the choice of the language that is used in the implementation of these algorithms, which need to be adopted and optimized applications. Learning for large scale of data, learning for different types of data, learning for high speed of streaming data, learning for uncertain and incomplete data, learning for data with low value density and meaning diversity are some of the important issue for future.

Challenges: Data capture and storage, Data transmission, Data curation (data quality assurance), Data visualization, Data analysis, are the issue which the scientists need to face in near future (Ed-Daoudy and Maalmi, 2019.)

To process the huge sized unstructured, inconsistent, incomplete and vague data generated by computing machines is a challenging task. To perform operations in the data, present in higher dimensions may be more computationally complex procedure as well as the computational overhead in further training and testing phases of classification (Xindong *et al.*, 2014.) In recent past years, Rough set theory and Fuzzy logic evolved as an efficient machine learning methodology, which has grown as an important tool to perform big data analytics.

Presently we are in a period of big data, preparing and investigation of huge

estimated, unstructured, conflicting, deficient and uncertain data which is a big testing assignment. To perform activity and investigate deficiency in a data, present in higher measurements is simply muddled or complex.

With the rise in big data, machine learning has become a key technique for solving problems in areas (Ruiz *et al.*, 2017) such as: automotive, aerospace, and manufacturing, for predictive maintenance, computational biology, for tumor detection, drug discovery and DNA sequencing, computational finance, for credit scoring and algorithmic trading, energy production, for price and load forecasting, image processing and computer vision, for face recognition, motion detection, and object detection, natural language processing, for voice recognition applications, and many more (Chen and Zhang, 2014, Ke and Shi, 2014.)

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